Primary complications after cardiac surgery: towards better understanding, prediction, and prevention

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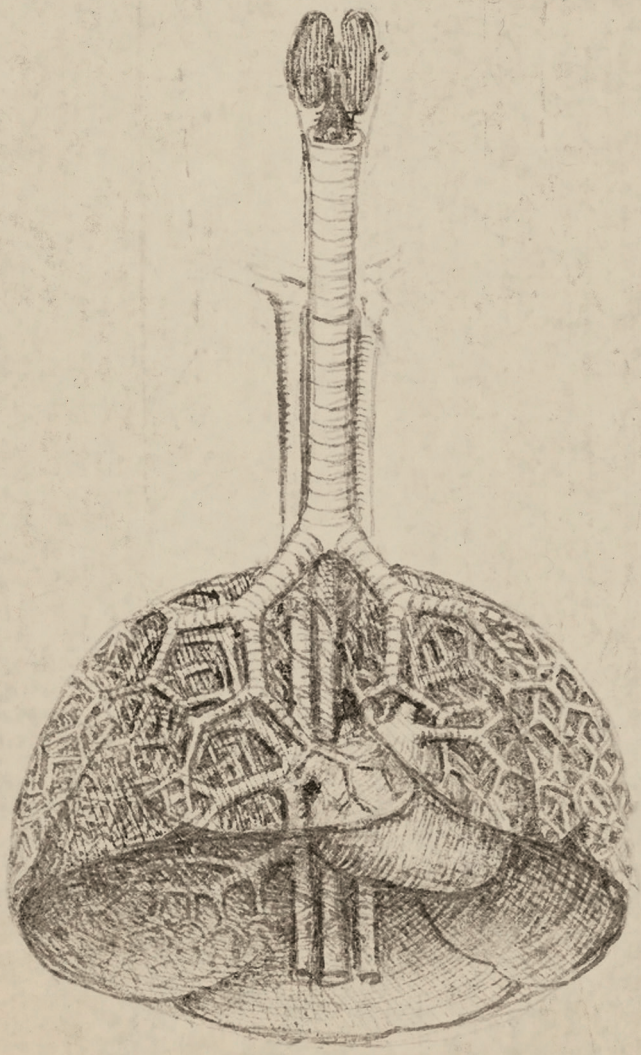
Pulmonary complications after cardiac surgery

Towards better understanding, prediction, and prevention



Judith van Paassen

Pulmonary complications after cardiac surgery

Towards better understanding, prediction, and prevention

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"… pulmonary problems remain the most significant cause of morbidity following cardiopulmonary bypass nowadays…"

Pennock JL, Pierce WS, Waldhausen JA.
Surg Gynecol obstet. 1977; 145(6);917-27

In almost fifty years not much has changed.

CONTENTS

General introduction
and outline of the thesis

PREFACE

Cardiac surgery and Inflammation

A frequent complication of cardiac surgery is the development of a systemic inflammatory response syndrome (SIRS). Several reports describe an incidence between 5 and 50% (1-3). Although the exact pathophysiology is not yet unraveled, important steps have been made that have deepened the knowledge and understanding of SIRS after cardiac surgery. Multiple factors lead to activation of the inflammatory response, of which the most important are the surgical trauma itself (4), direct contact of blood with the synthetic surfaces of the cardio-pulmonary bypass (CPB), ischemia-reperfusion injury, and blood transfusion (5). Surgical injury induced release of mitochondrial damage-associated molecular patterns (DAMPs) (6-8), complement activation through classical-, lectin- (9), and alternative pathways (7, 10-12), the subsequent or concurrent release of proinflammatory cytokines (Tumor Necrosis Factor(TNF)-α, Interleukin (IL)-1beta, IL-2, IL-6, IL-8) (7, 12), and anti-inflammatory cytokines (IL-10, IL-1RA, TNFsr1 and 2, and Transforming Growth Factor (TGF)-beta) (7), and the activation of platelets and neutrophils (resulting in rolling, tethering, endothelial -induced adhesion and -transmigration) (7, 13), result in endothelial injury. As a consequence of this inflammatory response, patients can develop multiple organ dysfunction with a complicated postoperative course and an unfavorable overall outcome (1-3).

ARDS

One of the most sensitive organs to the effects of SIRS are the lungs. As a result of the dysregulated systemic inflammation, a local inflammatory response in the lung itself starts and leads to development of acute lung injury and even of an adult respiratory distress syndrome (ARDS) (14). ARDS was first described in 1967 by Asbaugh et al. (15).

In order to overcome issues regarding the reliability and validity of the AECC ARDS definition (16), the Berlin ARDS definition task force came to a new definition of ARDS in 2012 (17), that is currently still in use and summarized in table 1. For the studies, i.e. the study populations, described in this thesis , the 2024 adjustments of the ARDS definition, pertaining to non-intubated patients and resource-limited countries, are of limited relevance (18).

There are over sixty defined causes of ARDS, but in all of them systemic inflammation plays a causal role in the development of local alveolar injury, typical for ARDS. DAMPs and neutrophils are attributed a key role in this process (19). DAMPs are recognized by Pattern Recognition Receptors (PRR), such as Toll like receptors (TLR), C-type Lectine receptors (CLR), RIG-1-like receptors (RLR) and NOD like receptors (NLR) These PRRs induce activation of a cascade of pro-inflammatory cytokines such as tumor necrosis

Table 1. Berlin ARDS definition

1.	Onset within 7 days after a known clinical insult or new or worsening respiratory symptoms		
2.	Bilateral opacities "that are consistent with pulmonary edema" on Chest X-ray or CT-scan, that are - not fully explained by effusion, atelectasis or masses - not exclusively due to a cardiac cause or fluid overload		
3.	Presence of an Identified risk factor for ARDS *If absent, an objective assessment (e.g. echocardiography) is necessary to exclude hydrostatic edema*		
4	Categorization of ARDS severity	MILD	PaO_2/FiO_2 = 201-300
		MODERATE	PaO_2/FiO_2 = 101-200
		SEVERE	$PaO_2/FiO_2 \leq 100$
5.	Minimum PEEP setting or CPAP: 5 cm H2O *PaO2/FiO2 setting assessed on mechanical ventilation; CPAP criterion used for the diagnosis of mild ARDS*		

CPAP continuous positive airway pressure; PEEP positive end expiratory pressure; $Pa0_2/FiO_2$: ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen

factor, and interleukins (IL) IL-1beta, IL-6, and IL-8 (20-23). Both locally activated and from out of the blood marginalized neutrophils (24) produce toxic substances through degranulation of proteases (19), and form neutrophil extracellular traps (NETs)(19). One of the four serine proteases present in neutrophil granules is Human Neutrophil Elastase (HNE) (25). HNE plays a pivotal role in microbial clearance. When extracellular HNE release is dysregulated, It can remodel the airways and lung parenchyma by (A) goblet cell metaplasia and increased mucine (Muc) production (MUC5AC, MUC4 and MUC1), and (B) promoting a sustained inflammatory response through TLRs and progressive damage of the alveolar-capillary membrane (20-23), leading to further capillary leakage and oncotic driven accumulation of protein-rich exudate in the alveoli (20, 26-28). Increase in interstitial fluid, combined with damage to the alveolar epithelium, eventually causes the air spaces to fill with proteinaceous edema and even bloody fluid and debris from degenerating cells. Loss of ability to upregulate alveolar fluid clearance and loss of functional surfactant (20, 29) further deteriorate pulmonary function and lead to impaired gas-exchange, decrease of compliance and pulmonary hypertension.

Although in one of the latest systematic reviews of randomized trials on this subject steroids seems to provide an effective approach to reduce the risk of death in ARDS patients (30), other pharmaceutical interventions for adults with ARDS have not been proven useful up to now, (31) and treatment of ARDS mostly comes down to supportive

care (32). Over the years the options for supportive care have been expanded with much more sophisticated mechanical ventilation strategies (32-35), neuromuscular blockade, (36) prone-positioning, (37) and the use of veno-venous Extra Corporeal Membrane Oxygenator (ECMO) in adult intensive care setting (38), and this has improved the outcome of ARDS patients (32).

ARDS and cardiac surgery

Cardiac surgery with the use of CPB is one of the well-known causes of development of acute lung injury and even of ARDS. The reported incidence varies from 1 to 8% (27, 39-41). The diagnosis ARDS in these patients is particularly difficult, since a certain extent of heart failure and hence cardiogenic pulmonary edema might also play a role in the clinical and radiologic picture (27). The cause of ARDS after cardiac surgery lies in the many successive and necessary actions and events, associated with open heart surgery, such as CPB, transfusion of blood products, ischemia reperfusion injury, mechanical ventilation, large volume shifts, and the direct surgical insult (27, 42).

In addition to these inevitable surgery related factors, concurrent presence of respiratory viruses, such as influenza, SARS-CoV-2, cytomegalovirus (CMV), herpes simplex virus (HSV), and respiratory syncytial virus (RSV) might also contribute to ARDS, since they are well known for their ability to cause disruption of the alveolar architecture by causing excessive inflammation (43). Indeed, development of COVID-19 in the postoperative phase of cardiac surgery patients is associated with adverse outcomes (44-46), and cardiac surgery in the influenza season is followed by an increased risk of developing ARDS, and prolonged dependency of mechanical ventilation, ICU- and hospital stay (47, 48).

Development of ARDS after cardiac surgery is associated with high mortality (15-80 %) (40, 42), and significant long term physical and psychological sequelae (42, 49). Considering the fact that worldwide >800,000 patients per year undergo coronary artery bypass grafting (CABG) surgery and approximately 150,000 patients undergo valve surgery (50, 51), ARDS undoubtedly represents a significant unmet medical need with profound economic implications (42).

RATIONALE OF THIS THESIS

To improve the outcome of the many cardiac surgery patients that trust their lives in the hands of cardiothoracic surgeons, anesthesiologists and intensivists, it is important to better understand, better predict, and, if possible, better prevent and treat the most severe pulmonary sequelae of cardiac surgery.

Increasing pathophysiological understanding might in multiple ways lead to improvement of pre- and peri-operative care by:

- Strengthening of current prediction models (EuroSCORE) (52): Expanding current risk models with inflammation markers that are more representative for the actual pulmonary state (53), will lead to better recognition of patients at risk for an unfavorable outcome.
- Guiding pre-operative therapeutic considerations: Recognition of patients at risk can guide therapeutic choices: one could reduce the complexity of the operation by limiting interventions, choosing a different type of valve, or even opting for a catheter-mediated procedure.
- Avoidance of preventable contributing factors: If viruses would appear to play a role in development of ARDS, preoperative screening or even vaccination could be a rational intervention.
- Adjusting perioperative management: Early identification of developing ARDS, could drive specific therapeutic decisions before, during and after surgery (e.g. Hb trigger and transfusion of blood products).

Understanding the risks for and the mechanisms leading to ARDS after cardiac surgery is therefore very important, and could be a first step towards personalized medicine (54), with interventions that offer best results for the specific patient.

THE OUTLINE OF THE THESIS

The aims of this thesis can be summarized in 3 sub-themes:

1. To increase the pathophysiological understanding of the development of lung injury, i.e. ARDS, after cardiac surgery.
2. To investigate whether biomarkers can predict lung injury after cardiac surgery.
3. To investigate whether prophylactic corticosteroids can improve (respiratory) outcomes in cardiac surgery and other ICU patients.

This thesis consists of 3 different parts, each part containing studies related to the different objectives, as formulated above.

Part one – Pathophysiology

With the studies in this part we aimed to gather insight in the pathophysiology of acute lung reaction after cardiac surgery. Perioperative alveolar inflammatory markers were assessed in relation to P/F ratio, a ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen, and a measure of lung injury. Furthermore, the role of an (asymptomatic) viral infection in relation to development of unfavorable outcome in cardiac surgery patients was investigated.

Broncho-alveolar biomarker profile

In the first study perioperative miniBAL samples were assessed for multiple markers of inflammation at the broncho-alveolar epithelium. In this way, the perioperative change of the pulmonary inflammatory cascade, as implicated in ARDS in general, was studied. The different types of white blood cells, IL-8, neutrophil degranulation protease Human Neutrophil Elastase (HNE), and their effect on MUC5AC and MUC5B, as representatives of the mucocilliary clearance (MCC) apparatus, were investigated with linear regression (ANOVA). Both their relation to P/F ratio and their commutual correlations were analyzed.

Asymptomatic viral infections

In the second and third study the role of respiratory viruses in the development of ARDS after cardiac surgery was investigated.

The second study was a retrospective cohort study of 2013 patients. We compared, duration of mechanical ventilation, the incidence of ARDS, length of ICU stay, and mortality in patients that underwent cardiac surgery in the Influenza season with patients that underwent cardiac surgery in the low/no influenza season, using multi variable analyses. In this study, we took the influenza season as a surrogate for respiratory virus infection, assuming that during the season there are more patients who subclinically had or during admission acquired respiratory viruses.

The third study, an observational prospective cohort study, explored the actual presence of respiratory viruses in cardiac surgery patients. All patients were free of symptoms, as pre-operatively screening was carried out by an anesthesiologist and a thoracic surgeon, and surgery would have been postponed when a respiratory tract infection was suspected. Viral load and the types of viruses present in the patients were recorded. Furthermore, presence of respiratory viruses was related to seasonality and clinical outcome parameters, such as P/F ratio, ventilation time and ICU stay.

Part two – Prediction

The next study, on an eighty-patient prospective cohort of cardiac surgery patients, describes the predictive pre-operative value of platelet and leukocyte activation, and their complex formation in the peripheral blood, as a possible expression of processes on the vascular endothelium side of the alveolar capillary junction. Activation patterns were compared between patients with a less favorable pulmonary outcome (i.e. a low <200 $Pa0_2/FiO_2$ (P/F) ratio) versus those with a better outcome (high > 200 P/F ratio).

In cardiac surgery prediction models are used to estimate patients' risk for adverse outcomes after cardiac surgery. The EuroSCORE is a frequently used model (52) and in recent studies on this subject it was suggested that incorporation of biomarkers in risk models is feasible and useful (54). Most risk scores, however, only weigh the pre-operative risk, but do not take into account the impact of surgery itself.

The fifth study, a prospective cohort study, focused on peri-operative pro-adreno-medullin (ProADM) increase to predict outcome. In particular, the additional value of pre-to-postoperative proADM-change to the EuroSCORE by multivariable logistic regression was assessed for prediction of ARDS and other clinical outcomes after cardiac surgery. Adding a "change over time during surgery"- marker to regular prediction scores, allows early identification of patients at risk for developing an ARDS or a protracted clinical course and enables targeted preventative interventions.

Part three – Prevention

To reduce SIRS after cardiac surgery, corticosteroid prophylaxis has been used for decades. A Cochrane meta-analysis (55), however, did not show a beneficial effect, nor did a large randomized DECS-trial on this subject (56, 57).

The first study in this part is a prospective cohort study, applying the instrumental variable analysis (IVA) by using the physician's preference as a pseudo-randomization instrument, to assess the effect of perioperative corticosteroid use on adverse outcomes in cardiac surgery patients. In this study the outcomes after the IVA were also compared to the outcomes in the DECS trial, that was published at the same time.

The second study is an update of our earlier systematic meta-analysis (55), and includes all RCTs published until March 2019. The primary aim of this review was to determine the effect of prophylactic corticosteroids on mortality in adult cardiac surgery with CPB. Secondary aims were to examine the effect of corticosteroids on complications of adult cardiac surgery, such as myocardial adverse events (including fatal and non-fatal myocardial infarction), pulmonary adverse events (including pulmonary oedema, infection, or prolonged postoperative ventilation for respiratory failure), atrial fibrillation, surgical site infection, gastrointestinal bleeding and duration of stay in the intensive care unit (ICU) and hospital.

The third and final study in this part was prompted by the special circumstance of the recent COVID-19 pandemic occupying ICUs with COVID patients. In that time, not much cardiac surgery was performed, and other important study questions were to be answered. Therefore, we conducted another meta-analysis on steroids in ARDS, but now, understandably, in a different study population. When this study was performed medical science was swamped by a tsunami of literature, often of varying quality and not seldomly retracted from even leading journals. During the initial waves of the COVID-19 pandemic different therapeutic interventions for COVID-19 patients followed each other in rapid succession, along with the evidence for these interventions. In the second wave, when the first promising results of the Recovery trial (58) began to circulate across the many (social) media channels, we conducted this meta-analysis to what is now de corner stone of COVID treatment at the ICU. Besides the the clinical effect of corticosteroids in COVID-19 patients on mortality, and need for mechanical ventilation, we also studied viral clearance, opportunistic infections, and antibiotic use.

CONCLUSIONS AND REFLECTION

After the three main parts the results of this thesis are summarized, followed by a discussion, general conclusions and future perspectives. Finally, the thesis is concluded with a Dutch summary, curriculum vitae, reference list and acknowledgments.

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PART ONE

Understanding lung injury after cardiac surgery

1

MUC5AC concentrations in lung lavage fluids are associated with acute lung injury after cardiac surgery

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ABSTRACT

Background: Heart surgery may be complicated by acute lung injury and adult respiratory distress syndrome. Expression and release of mucins MUC5AC and MUC5B in the lungs has been reported to be increased in acute lung injury. Aim of our study was to (1) investigate the perioperative changes of MUC5AC, MUC5B and other biomarkers in mini-bronchoalveolar lavage (miniBAL), and (2) relate these to clinical outcomes after cardiac surgery.

Methods: In this prospective cohort study in 49 adult cardiac surgery patients pre- and post-surgery non-fiberscopic miniBAL fluids were analyzed for MUC5AC, MUC5B, IL-8, human neutrophil elastase (HNE), and neutrophils.

Results: All measured biomarkers increased during surgery. Perioperative MUC5AC-change showed a significant negative association with postoperative P/F ratio (p=0.018), and a positive association with ICU stay (p=0.027).

Conclusion: Development of lung injury after cardiac surgery and prolonged ICU stay are associated with an early increase of MUC5AC as detected in mini-BAL.

BACKGROUND

Development of acute respiratory distress syndrome (ARDS) is a complication following cardiac surgery that is reported in 1-8 % of patients (1,2). ARDS is associated with a complicated postoperative course, e.g. prolonged duration of mechanical ventilation and of intensive care unit (ICU)- and in-hospital length of stay (3,4), high mortality (50-90%) (1,3,5), and significant long term physical and psychological sequelae (6).

ARDS is characterized by inflammatory injury to the alveolar-capillary barrier, and in ARDS following cardiac surgery, this is thought to be induced by the systemic inflammatory response syndrome (SIRS) that occurs in up to 40% of cardiac surgery patients (7,8). Activation of several inflammatory pathways plays an important role in the pathogenesis. Pathogen- and damage associated molecular patterns (PAMPs and DAMPs), complement activation, and release of pro- and anti-inflammatory cytokines lead to activation of platelets and neutrophils (9-13). Recognition of PAMPs and DAMPs by Pattern Recognition Receptors (PRP) leads to IL-1β and IL-8 driven recruitment of activated neutrophils into the alveolar space, where they degranulate and may form neutrophil extracellular traps (NETs), inducing an extravascular alveolar inflammatory cascade (14-16).

Human Neutrophil Elastase (HNE) is a predominant serine protease released by degranulating neutrophils. Whereas intracellular HNE is implicated in microbial clearance of ingested bacteria, it may also be released during degranulation. Excessive release may cause local tissue injury, leading to further capillary leakage and accumulation of protein-rich exudate (1,17,18). Furthermore, HNE is a powerful secretagogue for mucus-producing goblet cells in the airway epithelium and submucosal glands, and increases the expression of mucin proteins, including the secretory, gel-forming mucins MUC5AC and MUC5B, that are implicated in the pathogenesis of asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and other pulmonary diseases (14-16,18). Up to now, it is unknown to which extent these broncho-alveolar inflammatory factors, and in particular the end products MUC5AC and MUC5B, are involved in the development of ARDS after cardiac surgery.

In this study, we hypothesized that mucin production may contribute to development of ARDS after cardiac surgery. Therefore, we aimed to investigate the perioperative changes of MUC5AC and MUC5B and other biomarkers in relation to the severity of postoperative lung injury and other clinical outcomes (ICU stay and hospital stay).

METHODS

Study design

From January 2014 to January 2018, an explorative prospective cohort study was performed at the ICU of a tertiary referral hospital, the Leiden University Medical Center in the Netherlands. The study was approved by the medical ethical committee (protocol P117-11), registered under Clinical Registration number: ICTRP: NTR 5314, 26-05-2015, and conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments (19). The STROBE checklist (20) was used to report this study [supplement Chapter 1.1].

Study population

Eligible patients were adults undergoing elective cardiac surgery. Written informed consent was obtained from all included patients the day before surgery. Exclusion criteria were inability to sign informed consent, being less than 18 years old, emergency operations, semi-surgical procedures (as transcatheter valve implantation and minimal invasive robot surgery), preoperative corticosteroid use, and participation in another study. Since pulmonary samples had to be processed immediately, inclusion was dependent on availability of research and laboratory personnel.

Samples size

A formal sample size estimation was not possible because no earlier studies with this research question in this study population were available. Therefore, we applied a pragmatic approach to include as many patients as possible in the years that were assigned to conduct this study.

Perioperative care

Patients were admitted to the thoracic surgery ward on the day before surgery. Perioperative care for cardiac surgery patients is standardized in the LUMC and follows a pre-established care path. All details regarding the pre-operative, intra-operative and post-operative care at thoracic ward, operating room and the ICU are available in supplement Chapter 1.2.

Data collection

All pre-, intra- and postoperative data and clinical parameters were obtained from the electronic patient database (EPD) system of the hospital. This EPD is used preoperatively, in the operating room and in the ICU. In the ICU, continuous hemodynamic and ventilation monitoring is performed and data recorded. Four times per day an arterial blood gas analysis is performed and more frequently on clinical indication.

Sample collection

In intubated patients, non-fiberscopic mini-bronchoalveolar lavages (miniBAL) were performed at two time points: pre-surgery after intubation (T1), and at ICU arrival (T2). Via the endotracheal tube a CombiCath® catheter was introduced and 10 ml NaCL 0.9% was instilled and aspirated again. Dwelling time was short because otherwise there was not enough yield with the small volumes used. The catheter was advanced to wedge. It is assumed that material from the airways of approximately 1.3 mm in diameter, i.e. the diameter of the catheter, was obtained. Due to small volumes of lavage fluid recovered after the miniBAL, it was not possible to perform all analyses in each sample (see also supplement Table S1.1). It was decided to start with mucin analyses, then HNE, and if sufficient material was left, also IL8 analyses.

Laboratory analyses

Samples were processed in the laboratory immediately upon collection. After mucolysis using Sputolysin Reagent [DTT] (Calbiochem, cat nr. 560000) and filtering (using a 100-micrometre filter) the cells and debris were separated by centrifugation for 10 min, at 1500 rpm and room temperature.

Cell counts and differentiation: The pelleted cells were resuspended in phosphate buffered saline (PBS) containing 1% (wt/vol) human serum albumin (HSA). To enable optimal cell differentials, the concentration was adjusted to a concentration of 0.2 x 106 cells/ml. The cytospins were stained with Quick-diff (Dade Int. Inc., Deerfield, IL, USA) and manual differential cell counts of eosinophils, neutrophils, lymphocytes, macrophages, and epithelial cells were performed. From each miniBAL sample, two slides were prepared and stained. In each slide, at least 100 nucleated cells were counted manually and expressed as a percentage of the total number of nucleated cells. Mean values of these percentages were used in the analyses. The remaining supernatant was collected and stored at minus 80°C and analyzed later in one reagent batch to limit inter-assay variation.

IL-8 and HNE: The levels of soluble HNE and IL-8 were determined in the supernatant using enzyme-linked immunosorbent assay (ELISA) techniques. For IL-8, a commercial kit using mouse anti-human IL-8 antibodies wase used (CLB; Amsterdam, the Netherlands) and for HNE rabbit anti-human HNE antibodies were used (21).

Mucins: To assess the levels of MUC5AC and MUC5B protein in the supernatant, a dot blot-based immunochemical assay using mouse-anti-MUC5AC and rabbit anti-MUC5B was used (22). Levels were expressed as arbitrary units/ml (AU/mL) calculated based on a standard line constructed using serially diluted sputum samples. More details regarding laboratory procedures are available in supplement Chapter 1.3.

Endpoints

In this this study we studied the relation of the perioperative change of MUC5AC, MUC5B, IL8, leukocytes, and HNE in respiratory secretions in adult patients undergoing cardiac surgery with the development of lung injury (by means of P/F ratio measurement) and other clinical outcomes (ICU stay and hospital stay).

The P/F ratio is an objective tool to identify acute hypoxemic respiratory failure when supplemental oxygen is being administered and serves as a measure of acute lung injury. The P/F ratio is used to score ARDS severity in the Berlin Definition of ARDS (23), which has been shown to be significantly associated with mortality (3). The P/F ratio was only calculated in intubated patients as the ratio between arterial pO2 (PaO2) and fraction of oxygen in the inspired air (FiO2).

Statistical analyses

Statistical analysis was carried out as planned in advance, before collection of the data. We used descriptive statistics (mean with SD, median with IQR, or absolute numbers with percentages) to assess baseline parameters: demographics, comorbidity, presurgical performance status, and surgical parameters.

The respiratory secretions variables that were not normally distributed, were log-transformed to allow further analyses. The perioperative change was calculated as log (T2/T1) for each biomarker. Correlation between perioperative biomarkers were analyzed using Pearson's correlations testing. In the presence of a hypothesized inflammatory cascade and in view of the explorative nature of this study, a Bonferroni correction was judged not necessary. Finally, we related the perioperative biomarker changes to P/F ratio at ICU arrival. If a perioperative change could not be calculated due to missing values, or if a P/F ratio was not available (in extubated patients) that patient was excluded from that specific analysis. For biomarkers differences at a p-value < 0.05 were considered significant.

In this study, potential sources of bias were expected, but due to the explorative nature and the expected small sample size, no statistical analyses were planned in advance to correct for them.

The statistical analyses were conducted using the SPSS (Statistical Package for the Social Sciences), release 25.0 (SPSS Inc., Chicago)

RESULTS

From January 2014 to January 2018 a total of 49 patients were included in this study. The flow chart is shown in Supplement Figure S1.1. In one patient no miniBAL could be performed because of being extubated before ICU-admission. In table 1 patient characteristic and major clinical outcomes are shown. Patients were predominantly male with a slightly elevated BMI, and the majority had a history of myocardial infarction, diabetes, smoking, and hypertension (Table 1). Five patients had a moderately to severely impaired left ventricular function. The surgical procedures varied from coronary artery bypass grafting (CABG) to more complex combined valve surgery. The "other procedures" were pericardiectomy, left-ventricular reconstructive surgery, and heart failure surgery. Seventeen patients developed ARDS post-surgery, of whom two (4%) severe ARDS according to Berlin criteria. Median ICU stay was less than 24 h. One patient did not survive due to an exacerbation of underlying inflammatory lung disease.

Table 1. Patient Characteristics and Outcome (n=49)

Demographic parameters	
Age (yr) (mean, SD)	66.4 ± 10.2
Gender (male) (n, %)	28 (57)
BMI (kg/m^2) (mean, SD)	26.3 ± 4.3
Other relevant clinical data (n, %)	
Myocardial infarction in history	10 (20)
Percutaneous Catheter Intervention in history	14 (29)
Thoracic surgery in history	3 (6)
Hypertension	26 (53)
Malignancy in history	4 (8)
Chronic kidney insufficiency	4 (8)
Chronic liver disease	1 (2)
Diabetes	12 (25)
COPD	6 (12)
Smoking	31(63)
Pack years (mean, SD)	23.1 $\pm$ 14.9
Forced Vital Capacity (%) (mean, SD)	3.7 $\pm$ 1.1
FEV_1/FVC (without beta2-agonist) (mean, SD)	74.2 $\pm$ 9.9

Table 1. Continued

Ante-Surgery performance state (n, %)	
ASA I	0 (0)
II	8 (16)
III	38 (78)
IV	3 (6)
LVEF good LVEF > 55%	29 (59)
Reasonable LVEF 40-55 %	15 (31)
Moderate LVEF 25-40%	3 (6)
Poor LVEF < 25%	2 (4)
EuroSCORE logistic (median, IQR)	6 (5.6-6.4)
Surgical parameters	
Surgical procedure	
CABG	18 (37)
CABG + single valve	4 (8)
CABG + multiple valve	1 (2)
Single valve	5 (10)
Multiple valve	4 (8)
Thoracic Aorta surgery (+/- valve +/- CABG)	12 (25)
Other	5 (10)
Surgical duration	
Surgery (hrs) (median, IQR)	6.5 (6.2-6.8)
Cardiopulmonary bypass (hrs) (median, IQR)	184.0 (127.9-195.1)
Aorta Clamp time (min) (median, IQR)	127.0 (118-136)
Intraoperative Steroid use (n,%)	9 (18)
Outcomes	
ARDS according to Berlin Definition (n, %)	
Mild	9 (18)
Moderate	6 (12)
Severe	2 (4)
Ventilation time (min) (median, IQR)	724.0 (145-1303)
Length of ICU stay (hrs) (median, IQR)	23.7 (20.7-27.0)
Length of Hospital stay (days) (median, IQR)	8.0 (3-13)
30 days mortality (n, %)	1 (2)

BMI = body mass index: PCI = percutaneous catheter intervention: COPD = chronic obstructive pulmonary disease: ASA = American society of anesthesiologists score: LVEF = left ventricular ejection fraction: CABG = Coronary Artery Bypass Grafting: ARDS = Adult respiratory distress syndrome: ICU = Intensive care unit.

Absolute values for the different biomarkers at T1 and T2 are given in table 2. Neutrophils, IL8, HNE, MUC5AC and MUC5B all increased peri-operatively.

Table 2. Values for neutrophils, IL-8, HNE, MUC5A and MUC5B pre-operatively after induction of anaesthesia (T1) and at ICU-admission (T2)

Biomarker	**Preoperative (T1)**		**ICU arrival (T2)**		
(available samples)	*Mean*	*SD*	*Mean*	*SD*	*Ratio T2/T1*
Neutrophil (%) (27)	25.0	(30.3)	39.2	(28.0)	1.6
IL-8 (pg/ml) (13)	3521	(17099)	8629	(25535)	2.5
HNE (39) (AU/ml)	210.3	(417.9)	403.79	(683.8)	1.9
MUC5B (AU/ml) (42)	55.88	(157.9)	256.2	(461.3)	4.6
MUC5AC (AU/ml) (43)	132.47	(345.2)	1164.6	(3271.7)	8.8

IL-8 = Interleukin 8; HNE = Human Neutrophil Elastase; MUC5B = Mucin 5B; MUC5AC = Mucin 5AC; AE/ml = Arbitrary Units/milliliter

For all biomarkers, the degree of perioperative change, measured as T2/T1, was tested for correlation with the P/F ratio as a continuous variable. A significant (p=0.018) negative correlation was found between MUC5AC and P/F ratio at ICU arrival (Figure 1a, Table 3). Furthermore, a positive correlation of the perioperative MUC5AC change with ICU length of stay was found (p=0.027) (Figure 1b, Table 3). No significant association with other clinical outcome parameters was observed for any of the other biomarkers (Table 3)

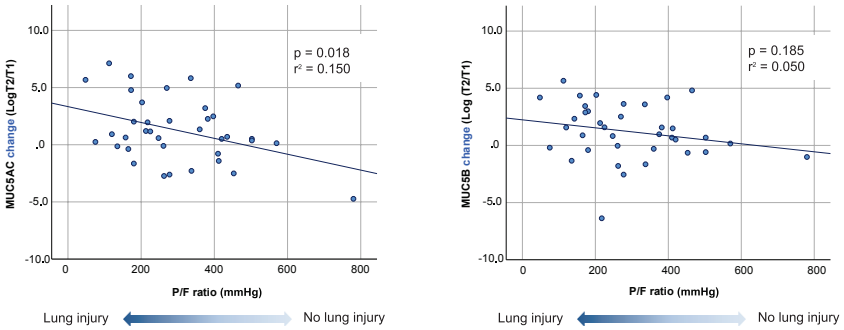
Table 3. Correlation between increase of biomarkers measured in miniBAL as concentration at T2 divided by concentration at T1 (T2/T1), and clinical outcomes.

	CPB-time	**Simple vs complex Surgery***	**Mechanical ventilation time**	**P/F ratio (mmHg)**	**ICU stay**	**Hospital stay**
Neutrophil (%)	0.048 (p=0.836)	0.016 (p=0.590)	0.310 (p=0.172)	-0.94 (p=0.685)	0.008 (p=0.971)	0.227 (p=0.323)
IL-8 (pg/ml)	0.176 (p=0.458)	0.015 (p=0.609)	0.240 (p=0.309)	0.155 (p=0.515)	0.229 (p=0.331)	-0.064 (p=0.789)
HNE (AU/ml)	-0.044 (p=0.786)	0.002 (p=0.772)	0.108 (p=0.500)	-0.219 (p=0.169)	0.141 (p=0.378)	-0.098 (p=0.554)
MUC5B (AU/ml)	0.073 (p=0.669)	0.080 (p=0.091)	0.209 (p=0.214)	-0.223 (p=0.185)	0.217 (p=0.197)	0.144 (p=0.402)
MUC5AC (AU/ml)	0.146 (p=0.387)	0.048 (p=0.194)	0.275 (p=0.100)	-0.388 (**p=0.018**)	0.362 (**p=0.027**)	0.289 (p=0.087)

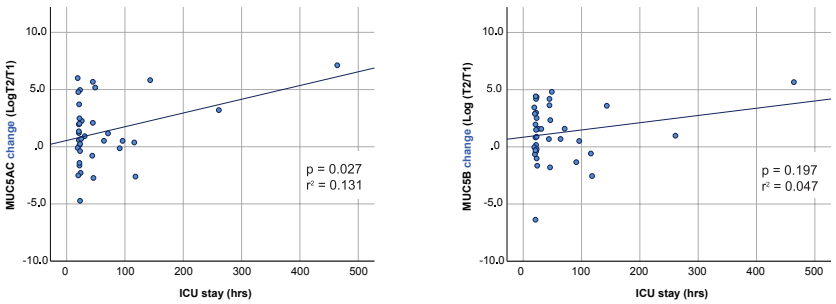
*Correlation with CPB-time, mechanical ventilation time, P/F ratio and ICU-LOS by Pearsons correlation coefficient. Correlation with type of surgery by ETA square correlation statistics. IL-8 = Interleukin 8; HNE = Human Neutrophil Elastase; MUC5B = Mucin 5B; MUC5AC = Mucin 5AC. CPB = cardiopulmonary bypass; ICU = intensive care unit *Simple: coronary artery bypass grafting with or without single valve surgery; Complex: all other surgery; AE/ml = Arbitrary Units/milliliter*

Figure 1. MUC5AC and MUC5B related to P/F ratio and ICU stay

a. Mucins related to P/F ratio



b. Mucins related to ICU stay



T1 = pre-operative timepoint; T2 = ICU arrival timepoint; P/F ratio = PaO2/FiO2 ratio.

DISCUSSION

In this study we show that concentrations of mucins in airway lavage fluid are increased peri-operatively from induction of anesthesia until admission to the ICU, and that the increase in MUC5AC is related to the severity of lung injury as measured by P/F ratio.

Not only mucins, but in fact all measured biomarkers increased peri-operatively. Thus, the increase of mucins may simply be one of many reflections of the activation of inflammation that takes place after cardiac surgery (24, 25). However, the magnitude of increase is five- to tenfold for MUC5B and MUC5AC and thereby much more than the increase of other biomarkers. Importantly, MUC5AC was the only biomarker that was significantly associated with relevant clinical endpoints, such as the severity of

lung injury and the length of stay in the ICU. Thus, we cannot rule out that mucins have an etiologic role in the development of lung injury and ARDS after heart surgery.

Our results are in line with one earlier study in children after cardiac surgery with cardiopulmonary bypass. In these children, MUC5B and MUC5AC in airway lavage fluid levels were significantly increased after surgery. Children with respiratory complications showed significantly higher MUC5AC levels than did children without respiratory complications and the increase of total mucin during cardiopulmonary bypass showed positive correlation with alveolo-arterial oxygen difference (26).

Mucins are major glycoprotein components of mucus and are important in pulmonary mucosal defense and the ability to resist lung injury. MUC5AC is produced in the superficial mucosa and MUC5B primarily in the submucosal glands (27). In normal airways, mucins cover the epithelial surface of the respiratory tract, and mucin production is maintained at a relatively low level to promote mucociliary clearance of inhaled and trapped substances. In pathologic conditions such as asthma, bronchitis, and acute respiratory distress syndrome, however, excessive mucus production limits mucociliary clearance, whereas mucus accumulation in the small peripheral airways may lead to mucus plugging and airway obstruction, ultimately impairing gas exchange (28). In critically ill patients with acute lung injury (ALI), MUC5AC levels in bronchoalveolar fluid were more than 58-fold increased (29). The concomitant elevation of the secretagogue HNE and the short time interval after start of surgery suggests a role for hypersecretion of mucin by already present goblet cells rather than upregulation of the number of goblet cells. The strong association between MUC5AC and lung injury does not imply that mucins play a direct etiologic role in acute lung injury. Alternatively, increased mucin expression could also be just a reflection of the proinflammatory state without a specific causal role. To better understand the importance of mucins, it would be interesting to study the influence of specific inhibition of MUC5AC in situations leading to acute lung injury. However, although various substances, such as azithromycin (30) and N-acetyl-cysteine (31), are known to inhibit the production of MUC5AC, it is unknown to which extent inhibition of mucin production contributes to their clinical benefit. Furthermore, studies using elastase inhibitors could provide more insight into the contribution of HNE-induced mucin production of lung injury following cardiac surgery. Recently, positive results of a phase 2 study using the elastase inhibitor Alvelestat in alpha-1 antitrypsin deficiency were reported in abstract form (32).

Some study limitations should be discussed. The fact that an association was found between MUC5AC and relevant clinical endpoints, but not for other biomarkers, may be caused by a lack of statistical power. Due to the small miniBAL samples of airway fluid, we were not able to test all samples for all biomarkers. It is well possible that

significant associations would have been found if more patients could have been tested for all biomarkers. As already discussed, the most important limitation is the impossibility to determine if mucins have a specific etiologic role in the development of lung injury, or that they should be considered as just some of the upregulated molecules of a neutrophil-dominated inflammatory cascade in the airways.

In conclusion, we show a marked increase in the concentrations of MUC5A and MUC5B in bronchoalveolar fluid of patients after heart surgery and a significant association between the increase of MUC5A and the severity of lung injury and ICU length of stay.

LIST OF ABBREVIATIONS

ARDS:	acute respiratory distress syndrome;
IL:	interleukin
HNE:	human neutrophil elastase
P/F ratio:	PaO2/FiO2 ratio
MUC:	mucin
ICU:	intensive care unit
SIRS:	systemic inflammatory response syndrome
PAMP:	pathogen associated molecular patterns
DAMP:	damage associated molecular patterns
NETs:	neutrophil extracellular traps
COPD:	chronic obstructive pulmonary disease
STROBE:	the strengthening the reporting of observational studies in epidemiology
EPD:	electronic patient dossier
PBS:	phosphate buffered saline
HSA:	human serum albumin
ELISA:	enzyme-linked immunosorbent assay
AU/ml:	arbitrary units/ml

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SUPPLEMENTARY MATERIAL

Contains:

2

Influenza season and ARDS after cardiac surgery

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INTRODUCTION

A pulmonary inflammatory response after cardiac surgery was described almost 60 years ago as a severe complication of such surgery (1). Still, this Acute Respiratory Distress Syndrome (ARDS) is considered life-threatening with a mortality rate of about 40 % of those affected (2,3). The pathogenesis of ARDS is complex and not all factors involved are elucidated (4). One “massive hit” may cause ARDS but it seems more likely that ARDS follows on multiple sequential minor insults (5,6). Cardiopulmonary bypass (CPB) might be the most important factor causing ARDS postoperatively (7). Transfusion of blood products, complexity of surgery and emergency procedures are additional insults or risk factors (3,8). Most of these factors are rigid and do not lend to intervention, to improve outcome after surgery.

Recently, it has become clear that symptomatic viral infections may cause acute lung injury. In particular, severe symptomatic influenza virus infection can lead to ARDS (9,10). Most influenza virus infections in adults, however, are asymptomatic. Thus, in a recent analysis, only 23 percent of influenza virus infections were symptomatic (11). Moreover, an asymptomatic respiratory virus infection has been demonstrated in a small cohort of patients undergoing elective cardiac surgery (12). Such asymptomatic infections could be a hit contributing to the development of ARDS, e.g. by inducing a low-grade inflammatory response in the lung priming this organ for ARDS in case additional insults would follow.

The aim of the present study was to fill in this deficit and to test in adults, respiratory virus infections as a risk factor for development of ARDS after cardiac surgery.

METHODS

Study design and study population

A single-center observational cohort study based on routinely collected clinical data was conducted at the Intensive Care Unit (ICU) of the Leiden University Medical Center (LUMC) between January 2009 and December 2011. This is a tertiary university hospital in the larger metropolitan area of the Netherlands. The ICU is a 25 bed, mixed medical, surgical, neurosurgical and thoracic surgical ICU. All patients ≥ 18 years of age were enrolled at admission on the ICU after cardiac surgery. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center.

Outcome measures

Primary outcome was ARDS within 7 days after cardiac surgery, according to the Berlin definition (13). Secondary outcomes were time on mechanical ventilation, length of stay in ICU and ICU mortality.

Perioperative care

Elective cardiac surgery patients were admitted to the hospital one day prior to surgery and were checked to be fit for surgery. In case of fever or clinically apparent respiratory tract infection on the day of surgery, the operation was postponed. A minority of patients underwent surgery in an emergency setting. These patients were not checked for fever or severe respiratory tract infections since their cardiac emergency was life-threatening and therefore warranted immediate surgery. Cardiac surgery involved coronary artery bypass grafting (CABG), ventricular surgery, valve surgery or aortic surgery. Induction of anesthesia was done with propofol and remifentanil. Anesthesia was maintained with midazolam or propofol and remifentanil and sufentanil. Tranexamic acid prophylaxis was given to minimize perioperative blood loss, cefazolin was used as antibiotic prophylaxis. CPB with a centrifugal blood pump was initiated. Oxygenation was ensured with a hollow fiber membrane oxygenator, tubing was coated with bio-inert heparin-free polymers. Flow was laminar. Intermittent warm antegrade blood cardioplegia was instituted. During CPB, heparin was used to achieve an activated clotting time > 400s. Patients were ventilated with low pressure and low tidal volume to prevent atelectasis of the lung, except for procedures in which persistent ventilation obstructed surgical procedures. During bypass, core temperature was maintained at 34°C to 36°C. Active cooling was solely used during aortic surgery to prevent brain ischemia. Inotropic and vasoactive agents were administered on indication. Perioperative care was according to the fast-track protocol (14,15). During the study period, no changes in protocols of intraoperative mechanical ventilation of cardiac surgery patients have been practiced in our institution. On the ICU, lung protective mechanical ventilation (PEEP 5-8 with small tidal volumes (<6ml/kg) according to the then prevailing mechanical ventilatory standards) has been used.

Demographic data, ASA category, EuroSCORE as preoperative risk assessment (16), APACHE IV score as a marker of severity of disease after surgery (17) were all recorded peri-operatively. Duration of surgery and duration of CPB were recorded. Administration of blood products was recorded.

Definition of ARDS

Patients were diagnosed with an ARDS if they have met each of the Berlin criteria (this thesis, page 2, table 1, nakijken of deze pagina nog klopt na final layout) (13). Cardiac surgery was the initial clinical insult. All criteria were measured within the first week after cardiac surgery. Chest roentgenograms were standardly obtained on admission on the intensive care unit (ICU) and 24 hours after admission to the ICU. If the respiratory condition of patients deteriorated, additional radiographic evaluation, including computer tomography (CT) scan, was performed. Presence or absence of bilateral opacities was judged by 3 independent observers on chest radiograph or CT scan. The observers were unaware of the study design. Consensus in interpretation was required. In case of discrepancy in radiograph or scan interpretation, uniformity was achieved by discussion between observers. Prominent hili on both sides were not considered as bilateral opacities. This feature is most likely due to cardiac failure and therefore not a feature of ARDS. Presence of hypoxia and ventilation prerequisites were monitored continuously throughout ICU stay. In case ARDS had developed, the point of time that the patient first fulfilled the Berlin criteria was recorded. The worst oxygenation during the first week after cardiac surgery was used to determine severity of ARDS.

Assessment of respiratory virus infection

Preoperative respiratory virus diagnostics is not routinely performed, and thus none of our patients was tested for presence of respiratory viruses. Of note, none of the electively operated patients experienced preoperative respiratory complaints or was febrile on admission which would have justified such diagnostics.

Therefore, we have used influenza season as a proxy for respiratory virus infection. Surveillance of influenza season is conducted by the Netherlands Institute for Health Services Research (NIVEL) and the WHO European Flu Network. They report the number of patients with influenza-like illness (ILI) in the Netherlands (18,19). NIVEL primary care database assembles 6 records from sentinel general practices in an information system. Data about ILI come from approximately 120,000 patients recorded in these GP practices and represent a good estimate of the Dutch population. ILI incidence was calculated per 100,000 persons per week between 2009 and 2011 (20). In temperate climate, infections with most respiratory viruses have a seasonal pattern (21-23) and therefore ILI incidence is fluctuating.

In the Netherlands, influenza epidemic season is defined by > 51 ILI-reports per 100,000 per week for at least two consecutive weeks and by the detection of Influenza virus in respiratory samples (24,25). An epidemic season ends in the first week ILI-reports fall to ≤ 51 per 100,000 per week. These patients with Influenza A or B virus infection contribute to a large extent to the seasonal increase in ILI numbers. The percentage of positive Influenza specimens is highest during the ILI epidemic season (26), making this the best indicator for Influenza virus activity (27).

In contrast to the well-defined influenza epidemic season, we have arbitrarily predefined a baseline season as at least two consecutive weeks < 25 ILI-reports per 100,000 per week. This represents a period with low incidence of respiratory virus infections. The baseline periods end in the first week ILI-reports increase to ≥25 per 100,000 per week. All other weeks were categorized as peri seasonal period. These periods represent build up phase to an epidemic season or a period of decreasing ILI activity towards a baseline season. Comparable baseline and peri seasonal periods in surveillance of respiratory viruses have been used previously (28).

Statistical analysis

Continuous variables were summarized as either means with standard deviations or medians with interquartile ranges. Categorical variables were depicted as numbers with percentages or as medians with interquartile ranges. Kruskal-Wallis and Chi-squared tests were performed for comparing baseline data as appropriate. Statistical analysis was performed using SPSS (IBM Software) version 23. To evaluate whether influenza epidemic season is a risk factor for development of ARDS or ICU mortality, we have used binary logistic regression to calculate odds ratio with 95% confidence intervals. For continuous secondary outcomes (time on mechanical ventilation and length of stay on ICU), we have used mixed linear modelling and calculated estimates with 95% confidence intervals. All variables that were significant ($p < 0.1$) in univariate analysis and variables that were deemed clinically relevant were entered in the multivariable logistic model. Emergency procedure or not was not added to the model since both EuroSCORE and APACHE IV score have this entity within their total score. EuroSCORE is used as preoperative risk assessment and therefore ASA category is not added to the model.

RESULTS

Between January 2009 and December 2011, in total 2021 patients have undergone cardiac surgery. Of these patients, 8 died during surgery and therefore 2013 were admitted to the ICU postoperatively. Of these patients, 289 have had surgery during an influenza epidemic season and 740 during a baseline period. The other 984 patients have had surgery during a peri seasonal period. The patient characteristics are shown in Table 1. Patients and research team had no influence on the season of surgery. No differences were found in demographic and perioperative variables between patients in the different seasons.

Table 1. Demographic data and perioperative details of studied population

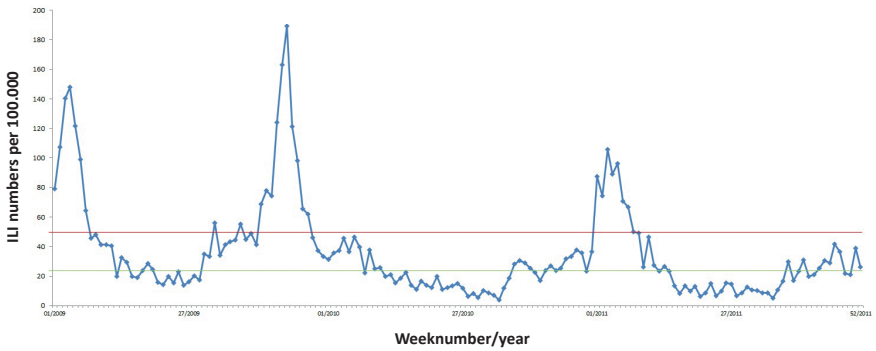
Variable	Baseline season	Peri seasonal	Influenza season	P Value **
All patients – no.	740	984	289	
Pre operative				
Age in years (IQR)	66 (58-74)	66 (58-75)	66 (58-73)	0.88
Gender- % males	72.2%	67.8%	67.1%	0.10
Body Mass Index- kg/m² (IQR)	26 (24-29)	26 (24-29)	26 (24-29)	0.92
ASA category (IQR)	3 (3-3)	3 (3-3)	3 (3-3)	0.68
Emergency procedure (%)	21 (2.8%)	27 (2.7%)	9 (3.1%)	0.95
EuroSCORE (IQR)	4 (2-9)	4 (2-9)	5 (2-9)	0.56
During surgery				
Duration of surgery – min (IQR)	360 (300-426)	358 (302-427)	354 (292-428)	0.81
Duration of CPB – min (IQR)	129 (99-184)	131 (94-185)	124 (92-183)	0.79
Units of blood products (IQR)	0 (0-1)	0 (0-1)	0 (0-1)	0.70
CABG (%)	435 (58.8)	597 (60.7)	167 (57.8)	0.59
Valve surgery	295 (39.9)	403 (41.0)	115 (39.8)	0.88
1 valve	195 (26.4)	279 (28.4)	73 (25.3)	
2 valves	91 (12.3)	106 (10.8)	34 (11.8)	
3-4 valves	9 (1.2)	18 (1.8)	8 (2.8)	
Aortic surgery (%)	80 (10.8)	109 (11.1)	39 (13.5)	0.45
Left ventricular reconstruction (%)	31 (4.2)	36 (3.7)	12 (4.2)	0.84
Postoperatively				
APACHE IV (IQR)	48 (37-59)	48 (38-60)	48 (39-61)	0.74
2nd Surgery required (%)	63 (8.5)	64 (6.5)	22 (7.6)	0.29

*IQR = Interquartile range; min = minute; no. = number *Medians are described for all continuous variables*
*** Kruskal-Wallis and Chi-squared tests were performed for comparison of baseline data as appropriate.*

The weeks with different influenza-like illness incidence are specified in Figure 1. Two influenza epidemic seasons were present in 2009. The first started in January and a second started in October. The subsequent year 2010 had no influenza epidemic period. In 2011, influenza epidemic season started in January.

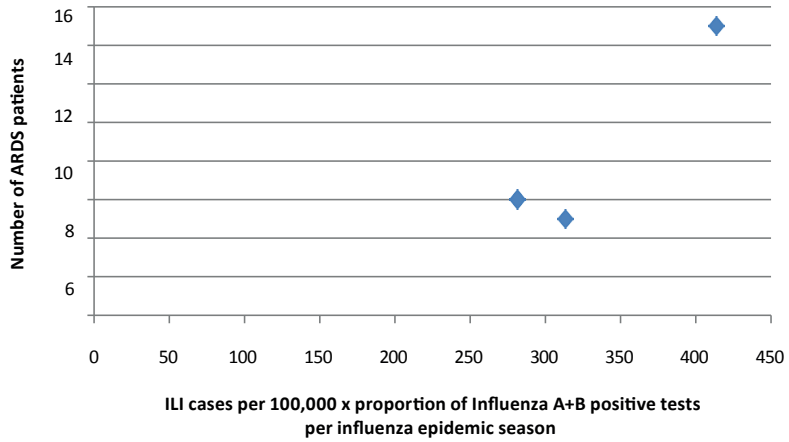
Of all patients who had been admitted on ICU after cardiac surgery in baseline weeks, 38 out of 740 (5.1%) developed ARDS. In peri seasonal period, 55 out of 984 (5.6%) developed ARDS and in influenza epidemic season 26 out of 289 (9.0%) developed ARDS. All ARDS cases occurred within 26 hours after start of surgery. Of all ARDS patients that underwent surgery during influenza epidemic season, 22 (85%) were admitted within 24 hours before surgery. (figure 2)

Figure 1. The Netherlands Institute for Health Services Research (NIVEL) reports its data on influenza-like illness (ILI) to the WHO European Influenza network.



Graphs depict ILI-reports between late 2008 until the end of 2011. The horizontal lines denote baseline (green) and epidemic (red) threshold within the studied period

Figure 2. ARDS and Influenza A+B per influenza epidemic season



In the univariate analysis (table 2A and 2B), the odds ratio for ARDS within 7 days after cardiac surgery in influenza epidemic season versus baseline season is 1.83 (95% CI 1.09-3.07).

Table 2A. Univariate analysis of demographic and perioperative parameters for ARDS and mortality

	ARDS within 7 days after surgery			Mortality on ICU		
	OR	95% CI	P	OR	95% CI	P
Age	1.00	0.98-1.01	0.52	1.02	0.99-1.04	0.22
Sexe	1.02	0.68-1.52	0.92	0.95	0.49-1.82	0.87
BMI	1.03	0.99-1.07	0.21	1.00	0.94-1.08	0.91
Apache IV	1.03	1.03-1.04	0.00	1.06	1.05-1.07	0.00
EuroSCORE	1.04	1.03-1.06	0.00	1.07	1.05-1.09	0.00
Time in surgery (min)	1.00	1.00-1.00	0.00	1.01	1.00-1.01	0.00
Blood products	1.07	1.04-1.10	0.00	1.10	1.06-1.14	0.00
Total time on CPB (min)	1.01	1.00-1.01	0.00	1.01	1.01-1.01	0.00
Emergency procedure	3.15	1.51-6.58	0.00	3.62	1.25-10.47	0.02
ASA 3*	4.21	1.32-13.40	0.02	3.39	0.46-25.09	0.23
ASA 4 and 5*	5.88	1.71-20.18	0.01	14.79	1.94-112.59	0.01
Periseasonal**	1.09	0.72-1.67	0.68	1.19	0.60-2.33	0.62
Influenza epidemic season**	1.83	1.09-3.07	0.02	1.48	0.61-3.56	0.39

** ASA 1 and 2 are the reference category. ** Baseline season is the reference category.*

Table 2B. Univariate analysis of demographic and perioperative parameters for time on mechanical ventilation and length of stay on ICU

	Time on Mechanical ventilation (min)			Length of stay on ICU (hours)		
	Estimate	95% CI	P	Estimate	95% CI	P
Age	0.39	-0.24-1.02	0.23	0.39	-0.26-1.04	0.24
Sexe	4.09	-12.21-20.38	0.62	4.43	-12.63-21.48	0.61
BMI	0.58	-1.20-2.36	0.53	0.50	-1.40-2.39	0.61
Apache IV	2.17	1.78-2.55	0.00	2.46	2.07-2.86	0.00
EuroSCORE	3.95	3.13-4.77	0.00	4.64	3.79-5.48	0.00
Time in surgery (min)	0.31	0.25-0.38	0.00	0.37	0.30-0.44	0.00
Blood products	8.66	6.71-10.60	0.00	11.74	9.69-13.80	0.00
Total time on CPB (min)	0.40	0.31-0.50	0.00	0.48	0.38-0.57	0.00
Emergency procedure	25.40	-18.85-69.64	0.26	35.13	-12.22-82.48	0.15
ASA 3*	19.32	-6.23-44.87	0.14	26.22	-0.11-52.54	0.05
ASA 4 and 5*	110.23	76.39-144.08	0.00	105.98	72.02-139.95	0.00
Periseasonal**	10.59	-5.73-26.92	0.20	5.83	-11.31-22.97	0.51
Influenza epidemic season**	27.16	3.83-50.50	0.02	25.11	0.59-49.63	0.05

** ASA 1 and 2 are the reference category.** Baseline season is the reference category.*

In the multivariate model (table 3A), odds ratio for ARDS in influenza epidemic season versus baseline season is 1.85 (95% CI 1.06-3.23). Furthermore, duration of mechanical ventilation was significantly increased in the influenza epidemic season compared to the baseline season (Table 3B). Other clinical outcome parameters did not differ significantly between baseline and influenza season. (Table 3A and 3B).

Table 3A. Multivariate analyses of demographic and perioperative parameters for development of ARDS and mortality on ICU

	ARDS within 7 days after surgery			Mortality on ICU		
	OR	**95% CI**	**P**	**OR**	**95% CI**	**P**
Apache IV	1.03	1.02-1.04	0.00	1.05	1.04-1.07	0.00
EuroSCORE	1.02	1.00-1.04	0.07	1.03	1.01-1.06	0.01
Time in surgery (min)	1.00	1.00-1.00	0.61	1.00	1.00-1.01	0.09
Blood products	1.00	0.95-1.05	0.93	1.01	0.95-1.08	0.76
Total time on CPB (min)	1.00	1.00-1.01	0.25	1.00	0.99-1.01	0.83
Peri seasonal*	1.09	0.69-1.72	0.71	0.96	0.43-2.11	0.91
Influenza epidemic season*	1.85	1.06-3.23	0.03	1.57	0.58-4.24	0.37

** Baseline season is the reference category*

Table 3B. Multivariate analyses of demographic and perioperative parameters for time on mechanical ventilation and length of stay on ICU

	Time on mechanical ventilation (hours)			Length of stay on ICU (hours)		
	Estimate	**95% CI**	**P**	**Estimate**	**95% CI**	**P**
Apache IV	1.61	1.20-2.02	0.00	1.79	1.37-2.20	0.00
EuroSCORE	1.58	0.64-2.51	0.00	1.69	0.77-2.62	0.00
Time in surgery (min)	0.19	0.06-0.31	0.00	0.13	0.02-0.24	0.02
Blood products	2.80	0.45-5.15	0.02	4.68	2.28-7.09	0.00
Total time on CPB (min)	0.00	-0.16-0.16	1.00	0.13	-0.02-0.27	0.08
Peri seasonal*	10.00	-5.49-25.49	0.21	6.51	-9.20-22.21	0.42
Influenza epidemic season*	22.64	0.47-44.81	0.05	21.08	-1.42-43.58	0.07

** Baseline season is the reference category.*

DISCUSSION

Cardiac surgery during influenza season is an independent risk factor for development of postoperative ARDS compared to surgery during seasons with little respiratory virus transmission.

The main finding of the present study is that the risk for the development of ARDS after cardiac surgery is about twice increased during the influenza season as compared to seasons with low burden of respiratory virus infections. Moreover, the influenza season did increase the duration of mechanical ventilation. The influenza season was estimated on the basis of weekly reporting of influenza-like illness within the community by sentinel surveillance at general practitioner offices, confirmed by detecting influenza in nasopharyngeal samples. On multivariate modelling, the influenza season proved to be an independent risk factor for the development of ARDS postoperatively, besides well-known factors like EuroSCORE and total time on CPB.

Our study has several strengths. Firstly, the database is robust and complete. The definitions used to define an influenza epidemic season and ARDS are widely used and accepted (13,18). The ARDS definition by the so-called Berlin criteria makes our results generalizable and readily permissible for comparison with other studies.

Surveillance of influenza-like illness is a good proxy for monitoring burden of influenza virus infection in the community (26,27). Since our hypothesis is that respiratory virus infection is an extra risk factor for developing ARDS in cardiac surgery patients, our study design is suitable to examine this expectation.

Although previous studies used different definitions of ARDS, the overall percentage of patients developing ARDS in our study (5.9%) resembles that of other studies (3,8,29-32).

The in-hospital mortality in patients with ARDS was 17%, which is somewhat less than that reported in other studies (2,3).

In recent literature, focus on moderate to severe ARDS instead of mild ARDS as a clinically relevant entity has come in use (33). Of note, in our cohort the influenza season was an even stronger risk factor for moderate to severe ARDS than it is for ARDS in general (data not shown).

Our study also has several weaknesses. First and for all, our cohort study shows an association but does not prove a causal relation between viral infection and ARDS in

cardiac surgery patients. There are potential confounders that vary by season, such as vitamin D level or ambient temperature, for which we could not adjust.

The 2009 Influenza A (H1N1) outbreak is remarkable in this aspect. The start of an influenza season in October is uncommon on the northern hemisphere. In this period of the year, temperature was higher than during usual epidemic periods in January to February. In addition, the vitamin D level shortly after summer should have been in the normal range, making these factors as being implicated in ARDS during influenza season less likely.

Still, we cannot exclude that such factors could have confounded our findings (34-36). Although surveillance of influenza season is robust, the 2009 H1N1 Influenza pandemic might have had an impact on the health seeking behavior of patients. For instance, fear for this new virus infection, might have lowered the threshold for visiting the general practitioner because of respiratory complaints (37,38) and the definition of influenza epidemic season might have been reached earlier. This bias could have underestimated our results.

The number of influenza seasons we studied, three, is too small to draw firm conclusions about secondary outcome variables and the post hoc analysis. Certainly, more influenza seasons with different products of ILI numbers and fraction of Influenza positive tests are required to determine the predictive value for ARDS.

How should we interpret the findings? Studies on the consequences of viral respiratory infection in cardiac surgery patients have mostly been done in the pediatric population. Children with upper respiratory tract infection or with documented rhinovirus infection at the time of cardiac surgery have more postoperative (respiratory) complications (39,40). On this evidence, it was suggested that an ongoing respiratory virus infection should influence the decision to postpone elective cardiac surgery in children (41). If latent respiratory virus infections are a risk factor for developing postoperative ARDS in adults as well, the chance of developing ARDS can be affected via this risk factor.

Spaeder et al. performed a study in children undergoing cardiac surgery. No differences in postoperative length of stay were detected between 'viral' and 'nonviral' season. However, in that study the definition of respiratory virus season was much more crude than in our study, their primary end point was different (length of stay) from ours and did not include ARDS, and the sample size was much smaller, making the study underpowered to reveal our association. Furthermore, children with symptomatic viral infections did have an increased risk of morbidity (42). Although viruses in children, their immune response and seasonality of these viruses are different from that in adults, a comparison of the hypothesis that viral infection is a predictor of worse (respiratory) outcome after surgery is reasonable. To our knowledge, our study is the first to assign respiratory virus season as a risk factor for ARDS in adult patients undergoing cardiac surgery.

H1N1 Influenza virus infections were predominant in the influenza outbreak in October 2009 (23,43). These infections are therefore presumably the most likely agents contributing to the increase in ARDS.

Our post hoc analysis is in line with this observation. Numbers of ARDS are higher during seasons with relatively more Influenza virus, defined as the ILI numbers multiplied by the proportion of Influenza A and B positive tests per week. This implicates the Influenza virus as the risk factor for ARDS more likely than other factors previously described. Of note, the patients undergoing cardiac surgery were at increased age and therefore less likely to acquire symptomatic H1N1 Influenza virus infection due to cross-reactivity against previously encountered H1N1 Influenza strains (44,45).

During influenza season, the percentage of positive swabs for other respiratory viruses is relatively high (23,46). Most likely, other respiratory viruses are equally important risk factors for the increased incidence of ARDS.

Of note, it is well known that symptomatic (H1N1) Influenza virus infection can be a cause of ARDS, with and without prior surgery. However, our study population differs from this group as it does not include patient with manifest respiratory (Influenza or not) virus infection.

Since most patients (97.2%) in our study have had elective surgery, they were checked preoperatively to assess whether they had an (acute) inflammatory disease. Surgery would have been postponed when infection was evident.

Our findings fit with the multiple hit hypothesis of ARDS pathogenesis. ARDS is most likely caused by multiple insults of which cardiac surgery and accompanying cardiopulmonary bypass are the most prominent. This study suggests that asymptomatic respiratory virus infection could prime the lungs for development of ARDS. The finding that ARDS occurs within 26 hours after cardiac surgery reflects our hypothesis that the lungs are primed, perioperatively, by viral infection.

Different studies support this theory.

A controlled randomized study in 1992 revealed that prophylactic antibiotics in patients undergoing aggressive antileukemic chemotherapy, reduced the number of sepsis and ARDS, most likely by removing streptococcal colonization from the upper airways (47). In a rodent model, a low-grade immune stimulus in the lungs before pneumonectomy caused aggravated lung injury in the contralateral lung compared to rodents who were not primed with the stimulus (48). The exact mechanism of this lung priming is not elucidated (49).

Previously, we demonstrated in a small cohort that in 18% of elective cardiac surgery patients, respiratory viruses could be detected in mini broncho-alveolar lavage (12). None of these patients had a manifest infection which is in line with a bigger cohort of patients with asymptomatic influenza virus infection (11).

Our population consists of – mostly elderly - cardiac patients, which should have received yearly immunization against influenza viruses. Of all Dutch patients with cardiac illnesses, 77.1% was vaccinated against influenza in 2011 (50). Therefore, our study design might be underestimating the association between influenza (season) and ARDS. On the other hand, the influenza vaccine effectiveness is less in older patients and might be insufficient to prevent subclinical influenza replication and infection (about 50% effective in preventing Influenza virus infection in the elderly) and, with that, ARDS (51). Other respiratory viruses with higher incidence during ILI season, for example RS virus, can also be the second hit in causing ARDS.

The majority of patients with ARDS in influenza epidemic season were admitted to the hospital ≤ 1 day before surgery. This essentially excludes nosocomial acquisition of influenza virus in most of the cases.

Future research is needed to test if vaccination of patients could reduce the risk of ARDS post cardiac surgery. Whether vaccination of health care workers could reduce this risk cannot be proven or ruled out by our study. Numbers are too small to draw firm conclusions.

In none of our cardiac surgery patients respiratory virus diagnostics were done before surgery. Remember that none had respiratory symptoms that might have justified such diagnostics. In the 7 days after cardiac surgery, in only 6 patients respiratory virus diagnostics were done. In none, a respiratory virus was detected, and none developed ARDS postoperatively.

Further research will be necessary to reproduce our findings and prospective studies to determine a causal relation is necessary. If confirmed, virus diagnostic testing or vaccination could be useful before high-risk cardiac surgery to attenuate the risk of postoperative ARDS.

LIST OF ABBREVIATIONS

ARDS:	acute respiratory distress syndrome
ASA:	American Society of Anesthesiologists classification
BMI:	Body Mass Index
CABG:	Coronary Artery Bypass Grafting
CPAP:	Continuous Positive Airway Pressure
CPB:	Cardio Pulmonary Bypass
ICU:	intensive care unit
ILI:	Influenza Like Illness
IQR:	Inter Quartile Range
NIVEL:	Nederlands Instituut voor onderzoek in de (Eerste Lijns) zorg
P/F ratio:	PaO2/FiO2 ratio
PEEP:	Positive End Expiratory Pressure
WHO:	World Health Organisation

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SUPPLEMENTARY MATERIAL

Non available

3

Viruses in the respiratory tract in patients undergoing elective cardiac surgery

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ABSTRACT

Objectives: Acute respiratory distress syndrome after cardiac surgery is a severe complication that is associated with high morbidity and mortality. The presence of viruses in the respiratory tract is postulated to be one of multiple factors attributing to development of acute respiratory distress syndrome (ARDS), but studies report conflicting results. Since this possible risk factor can potentially be influenced by screening or vaccination, we aimed to further investigate the role of viruses in the development of ARDS after cardiac surgery.

Methods: We conducted an explorative prospective cohort study in 49 randomly chosen asymptomatic adult elective cardiac surgery patients. On four different time points, non-fiberscopic mini-broncho alveolar lavages (miniBAL) were collected and analysed with multiplex PCR testing for 11 types of respiratory viruses.

Results and Conclusions: Various (merely low pathogenic) respiratory viruses were detected in 12% of our study population. Respiratory viruses were present both within and out of the influenza-like-illness- season. 19 (39%) of all patients developed acute respiratory distress syndrome. No relationship of viral presence with major pulmonary outcomes (PaO_2/FiO_2 ratio, development of acute respiratory distress syndrome or mechanical ventilation time) could be demonstrated, though events were too few to allow multivariate analyses.

In conclusion: Asymptomatic elective cardiac surgery patients do carry respiratory viruses, though not associated with development of respiratory complications. Further research is warranted, in particular research into the (more pathogenic) diverse subtypes of the respiratory viruses, the relevance of virus load (cycle threshold-values) and even into the diagnostic method (throat swab versus deeper material).

INTRODUCTION

Development of mild to severe acute respiratory distress syndrome (ARDS) after cardiac surgery occurs in 0.4-20% of patients (1-4). Severe ARDS is associated with a complicated postoperative course with e.g. prolonged mechanical ventilation time, intensive care unit (ICU)- and hospital stay (2-8), and high mortality (50-90%) (4-8). It is hypothesised that ARDS in these patients results from sequential events that lead to an inflammatory response with disruption of the alveolar wall (that comprises alveolar epithelial cells and capillary endothelial cells) and progressive alveolar oedema with disturbed gas exchange (9-11). Endothelial injury by the systemic inflammatory response due to surgery itself, cardiopulmonary bypass (CPB) and blood transfusion, (11) could be one of these factors, while the mode of mechanical ventilation (12), and collapse of the lung to facilitate the surgeon (13,14), could additionally also disrupt the epithelium of the alveolar wall.

Another factor that can influence alveolar integrity is perioperative airway colonisation with potentially pathogenic microorganisms, such as respiratory and herpes viruses. As an example influenza, SARS-CoV-2, cytomegalovirus (CMV), herpes simplex virus (HSV) and respiratory syncytial virus (RSV) are all known for their ability to cause disruption of the alveolar architecture by causing excessive inflammation (15). In transthoracic esophagectomy- and lung cancer surgery patients, presence of viral RNA/DNA indeed was found to be associated with postoperative pulmonary complications (16-18). Development of COVID-19 in the postoperative phase of cardiac surgery patients is also associated with adverse outcomes (19,20). Moreover, cardiac surgery in the influenza season is followed by an increased risk of developing ARDS (9% versus 5%) and prolonged dependency of mechanical ventilation, ICU- and hospital stay (9,21,22). Therefore, further knowledge on the role of respiratory viruses in development of ARDS after cardiac surgery is required, since, if established, it could lead to relevant preventive measures, such as rapid PCR screening and/or even targeted vaccination strategies in the pre-operative period.

In this prospective cohort study, we therefore investigated whether perioperative evidence of respiratory tract viral RNA/DNA in elective cardiac surgery patients is associated with adverse postoperative pulmonary outcomes.

MATERIALS AND METHODS

Study design and study population

From January 2014 to December 2018, a prospective cohort study was performed in a 26-bed ICU of the Leiden University Medical Centre at Leiden (LUMC), a tertiary referral hospital in the Netherlands. The study was approved by the Medical Ethical Committee (protocol P117-11) and conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments (23).
Eligible patients were adults (> 18 years) undergoing elective cardiac surgery. Although approximately 1000 cardiac surgeries were performed each year, the number of inclusions was dependent on laboratory and staff availability. The exclusion criteria were inability to sign informed consent, emergency operations, and participation in another study. Written informed consent was obtained from all included patients on the day before surgery.

Perioperative care

All patients visited the pre-operative outpatient clinic for pre-operative screening. At admittance, all patients were seen by an anaesthesiologist and a thoracic surgeon on the day before surgery. If there was the suspicion of an active infection on the basis of respiratory symptoms or elevated temperature, it was customary to postpone the operation. Perioperative care for cardiac surgery patients is standardized in the LUMC and follows a pre-established care path. All details regarding the pre-operative, peri-operative and post-operative care at thoracic ward, operating room and the ICU are summarized in supplement Chapter 3.1.

Data collection

All pre-, intra- and postoperative data and clinical parameters were obtained from the electronic patient database (EPD) system of the hospital. On the ICU, continuous hemodynamic and ventilation monitoring was recorded. Four times per day arterial blood gas analysis was performed and more frequently on indication. The age adjusted Charlson Comorbidity Index (aCCI), a prognostic score summing the weighted scores of 19 medical conditions, taking the seriousness and number of comorbid diseases into account, was calculated (24). This score is developed to predict 1 year-mortality based on medical comorbid conditions and age, and has since been validated in many different populations (25,26).

Influenza Like Illnesses (ILI) season

Ever since 1970, the Dutch national knowledge institute for primary health care research keeps track of when the ILI and Influenza season starts and ends (27). Predefined “index” general practices register the number of patients that report with ILI and standardised weekly tests on respiratory viruses are performed in these practices to define, by extrapolation, the number of new ILI cases/100.000 inhabitants. The ILI season is defined to last as long as this number of new patients is above 51/100.000 inhabitants per week.

Sample collection

On four different time points, non-fiberscopic mini-broncho alveolar lavages (miniBAL) were performed after intubation and induction, on the first, third and fifth postoperative day, but only if patients were still intubated. Briefly, 10 ml NaCl 0,9% was instilled via a Combicath® catheter and aspirated again. Samples were processed in the laboratory immediately. After mucolysis using Sputolysin Reagent [DTT] (Calbiochem, cat nr. 560000, Darmstadt, Germany and/or its affiliates), the cells and debris was separated by centrifugation. The remaining supernatant was collected and stored in 5 to 7 aliquots of 1 ml at minus 80 degrees Celsius.

PCR testing

After completing miniBAL sample collection and processing, the stored supernatants of all patients were tested at the same time, with the same batch for viral RNA/DNA. A multiplex real-time PCR (qPCR) developed in our laboratory was used, consisting of four types human corona viridae (hCoV) (229E, HKU1, NL63, and OC43), influenza virus type A (IVA) , influenza virus type B (IVB), human metapneumovirus (hMPV), parainfluenza (PIV) 1–4, respiratory syncytial virus (RSV), rhinovirus (RV), adenovirus, bocavirus, cytomegalovirus (CMV), herpes simplex virus (HSV) 1-2 (27-30). Total nucleic acids were extracted directly from 200 µl clinical samples, using the Total Nucleic Acid extraction kit on the MagnaPure LC system (Roche Diagnostics, Almere, the Netherlands) with 100 µL output eluate. Nucleic acid amplification and detection by real-time PCR was performed on a BioRad CFX96 thermocycler, using primers, probes and conditions as described previously (28,30). The cycle threshold (CT) value is the number of cycles of amplification required for a positive signal, measured as a curve above a threshold. CT-values were normalised using a fixed baseline fluorescence threshold. To prevent false positive results due to technical laboratory issues, such as artefact fluorescention or contamination, CT-values > 40 were considered negative.

Endpoints

The primary endpoint is the presence of respiratory viruses in adult elective asymptomatic cardiac surgery patients. The secondary endpoints were to assess whether perioperative presence of viral RNA/DNA in the respiratory tract is associated with clinical outcomes, such as PaO_2/FIO_2 ratio (P/F ratio: formula in supplement Chapter 3.2), Alveolar-arterial gradient (A-a gradient: formula in supplement Chapter 3.2), development of ARDS according to Berlin criteria (31). (definition in supplement

Chapter 3.3 of verwijzen naar inleading en uit supplement halen), and duration of mechanical ventilation and length of ICU stay.

Statistical analyses

Normal distribution of the data was tested according to Shapiro-Wilk. Mean and SD were used to describe normally distributed variables. Median and interquartile range (IQR) were used to describe non normally distributed variables. For the comparison of normally distributed variables, a Welch two sample t-test was used for two group comparison, and an analysis of variance (ANOVA) test for more than two groups. Non normally distributed variables were compared using the Mann-Whitney U test for two-group comparisons and the Kruskal Wallis test for more than two groups. Categorical variables were described as a count and a percentage. Given the small sample size, especially in the viral RNA/DNA group, we used Fisher's exact test rather than the Chi-squared test to compare categorical variables. Statistical analysis was performed using R Statistical Software (version 4.0.5).

RESULTS

Patient characteristics

From January 2014 to December 2018, 49 random patients were included in this study, six of whom had postoperative evidence of respiratory viral RNA/DNA at the day of surgery. In two patients it took more than 40 PCR cycles to detect viral genetic material, and they were considered virus negative. The patient characteristics are shown in table 1. There were more male patients (83 versus 54%), and less comorbidity (aCCI of 2.7 (SD 1.5) versus 3.7 (SD 1.6)) in the virus versus the non-virus patient group. In both groups surgery took place in the ILI season in the majority of patients (virus: 67% versus non-virus: 60%). Furthermore, there were less patients with heart failure, defined as an left ventricular ejection fraction of < 40 % (virus: 0 (0%) versus non-virus: 5 (11%)). COPD (GOLD 1 and 2) was more frequent in the non-virus group as well. In general, a high risk population was included, as evidenced by the high NYHA score, the pre-surgery risk score (EuroSCORE (32)), and the limited number of solely coronary artery bypass grafting (CABG) surgery. Surgery was more complex with more combined interventions in the non-virus group, but EuroSCORE, CPB time, and surgery time were not significantly different.

Table 1. Patient Characteristics

		Respiratory virus present in miniBAL		
		NO (n=43)	**YES (n=6)**	**p value**
Demographic characteristics				
Gender – Male	n (%)	23 (53)	5 (83)	0.22
Age (years)	Median (IQR)	68 (61 to 77)	66 (57 to 67)	0.15
BMI (kg/m2)	Mean (SD)	27 (5)	26 (3)	0.83
Surgery in influenza-like illness season	n (%)	26 (60)	4 (67)	1.00
Pulmonary condition				
Smoking status				
Current smoker	n (%)	5 (12)	0 (0)	1.00
Former smoker	n (%)	21 (49)	4 (67)	
Never	n (%)	15 (35)	2 (33)	
Packyears (years)	Median (IQR)	27 (14 to 39)	45 (38 to 53)	0.18
COPD				
NO	n (%)	36 (84)	6 (100)	1.00
GOLD 1	n (%)	4 (9)	0 (0)	
GOLD 2	n (%)	2 (5)	0 (0)	
GOLD 3	n (%)	0 (0)	0 (0)	
GOLD 4	n (%)	0 (0)	0 (0)	
Cardiac condition				
Clinical signs of decompensation	n (%)	2 (5)	0 (0)	1.00
Myocardial infarction in history	n (%)	8 (19)	2 (33)	0.59
PCI in history	n (%)	12 (28)	2 (33)	1.00
Left ventricle systolic function				
Good (EF > 55%)	n (%)	27 (63)	2 (33)	0.31
Moderate (EF 40%- 55%)	n (%)	11 (26)	4 (67)	
Impaired (EF 25%- 40%)	n (%)	3 (7)	0 (0)	
Poor (EF <25%)	n (%)	2 (5)	0 (0.0)	
NYHA[g] class				
Class I	n (%)	8 (19)	0 (0.0)	1.00
Class II	n (%)	7 (16)	1 (17)	
Class III	n (%)	14 (33)	1 (17)	
Class IV	n (%)	2 (5)	0 (0)	

Table 1. Continued

		Respiratory virus present in miniBAL		
		NO (n=43)	YES (n=6)	p value
Other comorbidities				
Diabetes Mellitus	n (%)	12 (28)	0 (0)	0.31
Hypertension	n (%)	23 (53)	3 (50)	1.00
Hypercholesterolaemia	n (%)	17 (40)	0 (0)	0.13
Malignancy	n (%)	0 (0)	4 (67)	1.00
Chronic liver disease	n (%)	1 (2)	0 (0)	1.00
Chronic kidney disease	n (%)	4 (9)	0 (0)	1.00
Preoperative creatinine (umol/L)	Median (IQR)	81 (67 to 96)	82 (78 to 92)	0.47
Age adjusted CCI	Mean (SD)	3.7 (1.6)	2.7 (1.5)	0.15
Surgical characteristics				
Euro SCORE 2	Mean (SD)	5.9 (2.8)	6.5 (1.5)	0.59
Type of surgery performed				
CABG	n (%)	17 (40)	2 (33)	0.85
CABG + single valve	n (%)	1 (2)	0 (0)	
CABG + multiple valve	n (%)	4 (9)	0 (0)	
Single valve	n (%)	2 (5)	0 (0)	
Multiple Valve	n (%)	4 (9)	0 (0)	
Thoracic Aorta Surgery ± CABG ± valve	n (%)	6 (14)	1 (17)	
Heart failure surgery	n (%)	9 (21)	3 (50.0)	
Total duration on CPB (minutes)	Mean (SD)	188 (89)	175 (51)	0.73
Total duration of surgery (minutes)	Mean (SD)	403 (128)	441 (111)	0.50
Intra-aortic balloon pump	n (%)	1 (2)	0 (0)	1.00
Erythrocyte transfusion (ml)	Median (IQR)	0 (0 to 0)	250 (63 to 625)	0.02
Fresh Frozen plasma transfusion (ml)	Median (IQR)	0 (0 to 0)	300 (0 to 600)	0.10

IQR interquartile range, BMI body mass index, SD standard Deviation, COPD chronic obstructive pulmonary disease, PCI Percutaneous coronary intervention, EF ejection fraction, NYHA New York heart association, CCI Charlson comorbidity index, CABG coronary artery bypass grafting, CPB cardiopulmonary bypass

Viral characteristics

In six out of 49 patients (12%) respiratory viruses could be detected (table 2). All patients were positive on the day of surgery itself. In one patient, two types of viral DNA were present (IAV and PIV type 2), and this patient was the only one to be still intubated on the first postoperative day. In this patient the CT value of IAV was lower in the second sample (decrease from 39.3 to 2 24.2), indicative of an increasing viral load as seen in an active infection. Mean CT values in the samples from the six positive patients were 37.2 (SD 2.1) in the ILI season showing no difference with 36.7 (SD 3.2) outside the ILI season (p = 0.84). Presence of viral RNA/DNA was similar in the ILI and outside the ILI season (4/30 in versus 2/19 outside the season) (p= 1.00).

Table 2. Virus Characteristics

Patient	Lowest CT value	Virus	Surgery in ILI[l] season	Timepoint virus positivity
1	24.15; 39.06	influenza A virus (IAV) parainfluenza virus (PIV-2)	yes	Surgery day and Day 1
2	38.28	parainfluenza virus (PIV-1)	yes	Surgery day
3	39.03	parainfluenza virus (PIV-2)	no	Surgery day
4	36.46	parainfluenza virus (PIV-3)	no	Surgery day
5	34.12	human Corona Virus (HCoV-HKU1)	yes	Surgery day
6	37.43	human Corona Virus (HCoV-HKU1)	yes	Surgery day

CT cycle threshold, ILI influenza like illnesses

Clinical outcomes in virus versus non-virus groups

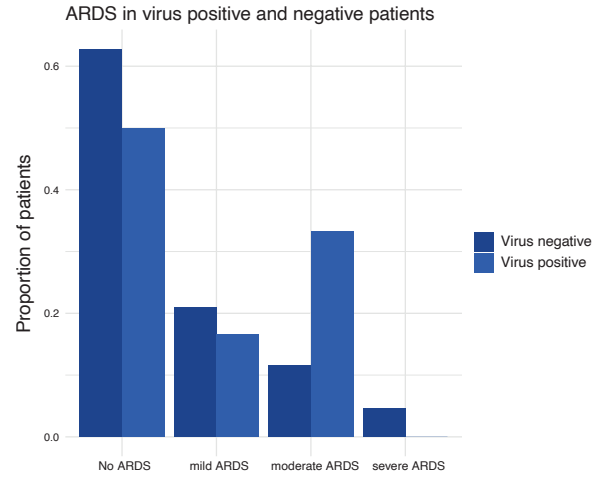
Pulmonary outcomes were not different in the virus versus the non-virus group (table 3). Neither the number of patients that developed ARDS, (virus: 3 (50%) versus non-virus: 16 (37%) (p = 0.51)), nor the severity of ARDS (virus: moderate/severe ARDS 33 % versus non-virus: 16% (p = 0.31)) were significantly different (figure 1). Duration of mechanical ventilation (min) (virus: 804 (637 – 990) versus non-virus: 767 (663 - 1227), p 0.70) and ICU stay (hours) (virus: 23 (21 - 39) versus non-virus: 24 (22 - 48, p = 0.50) were similar in both groups. Furthermore, more severe complications were seen in the non-virus group, though absolute numbers are small (table 3).

Table 3. Clinical outcome in virus versus non-virus groups

		Respiratory virus present in miniBAL		
		YES (n=6)	**NO (n=43)**	**p value**
Pulmonary outcomes				
Lowest P/F ratio after surgery	Median (IQR)	274 (199-349)	188 (139-267)	0.15
Lowest A-a gradient after surgery	Median (IQR)	0.8 (0.7-0.9)	0.9 (0.6-1.5)	0.52
ARDS				
No ARDS	n (%)	3 (50)	27 (63)	0.51
mild ARDS	n (%)	1 (16)	9 (21)	
moderate ARDS	n (%)	2 (33)	5 (12)	
severe ARDS	n (%)	0 (0)	2 (5)	
Mechanical ventilation time (min)	Median (IQR)	804 (637-990)	767 (663-1227)	0.70
Reintubation	n (%)	0 (0.0)	5 (12)	1.00
Severe complications				
Re-thoracotomy	n (%)	0 (0.0)	5 (12)	1.00
Cardiopulmonary resuscitation	n (%)	0 (0.0)	1 (2)	1.00
Cardiac tamponade	n (%)	0 (0.0)	3 (7)	1.00
Mortality	n (%)	0 (0.0)	2 (5)	1.00
Other				
Infection	n (%)	0 (0.0)	4 (9)	1.00
Delirium	n (%)	1 (16.7)	9 (21)	1.00
ICU stay in hours	Median (IQR)	23 (21-39)	24 (22-48)	0.50
ICU readmission	n (%)	0 (0)	1 (2)	1.00

IQR interquartile range, P/F ratio PaO_2/FiO_2 ratio, A-a gradient Alveolar-arterial oxygen gradient, ARDS adult respiratory distress syndrome, ICU intensive care unit.

Figure 1. Development of ARDS in virus and non-virus groups (p = 0.510)



DISCUSSION

In this explorative prospective cohort study of randomly chosen adult asymptomatic cardiac surgery patients, we demonstrated the presence of respiratory viral RNA/DNA in six out of 49 patients (12%). In these six patients, the viral RNA/DNA titre was generally low (high CT-value) and did not differ in or out of the respiratory virus season. Although moderate to severe ARDS occurred more often in the virus group (33 %) than in the non-virus group (16%), a significant association of respiratory tract viral RNA/DNA with unfavourable (pulmonary) outcomes, could not be established.

Previous studies reported variably on the clinical impact of the presence of respiratory viruses on surgery outcomes. Three large studies showed that the ILI season was unfavourably linked to clinical outcomes after surgery (9,21,22). However, in these studies no direct proof was provided that a viral infection was the causal factor in adverse outcomes following cardiac surgery. In fact, in our study, respiratory viruses were detected both in and out of the ILI season, with no significant differences in pulmonary outcomes. This virus independent occurrence of ARDS is consistent with paediatric studies of respiratory viruses in immunocompetent cardiac surgery patients (29,33). Moreover, in a large (n=1407) multicentre Dutch cohort of acutely admitted adults requiring invasive mechanical ventilation (also including 4% adult cardiothoracic patients) with 28 days of follow up, presence of virus was not associated with the number of ICU free days nor with crude mortality (34).

For all that, within the ILI season, the number of circulating respiratory viruses, and thus the risk of viral infection, remains undoubtedly higher. The preoperative assessment by the thoracic surgeon and anaesthesiologist, however, clearly succeeded in allowing only patients for surgery that showed no clinical signs of viral infections. Nevertheless, as our results indicate, patients with very low viral loads, hence subclinical viral infections, do undergo surgery. However, and notwithstanding their low number, we found this not to be associated with an unfavourable clinical outcome compared to elective patients without presence of respiratory viruses. This result should of course be interpreted with caution. While more non pulmonary complications occurred in the non-virus group, we cannot rule out the possibility that, in the virus group, this might have masked a possible effect of the virus itself, and multivariate correction was only possible to a limited extent due to the small sample size. While earlier studies suggested to include viral screening in the preoperative assessment (and possibly even vaccination) to prevent pulmonary complications after cardiac surgery, our study suggests that surgery on patients without symptoms of a viral infection is safe. Our results hence do not support a policy to include PCR-based preoperative screening for respiratory viruses in asymptomatic patients and/or postpone surgery in all asymptomatic patients with preoperatively detectable virus RNA/DNA in respiratory samples, though the heterogeneity of the data and small sample size make that results are difficult to interpret.

Screening for high CT values (low viral loads) of selected respiratory viruses - irrespective of the season - might still contribute to predict unfavourable outcomes. Some of these viruses (IVA, IVB (9,21,22), RSV (29,30,33), PIV (35), SARS-CoV-2 (36,37), HSV (38-40), and CMV (41)) are more pathogenic and can in themselves lead to the development of viral pneumonitis. Since our panel of viruses also included low pathogenic viruses, such as HCoV 229E, NL63, HKU1, OC43, PIV1-4, hMPV, and RV, we found a variety of different viruses in our patients of which there were only four that were associated with an unfavourable outcome in other studies. Hence, future research into higher pathogenic viruses and ARDS after cardiac surgery patients remains useful. Furthermore, there was only one patient with a CT value lower than 35. This could be indicative of a very early stage of the infectious process or, just the opposite, be indicative of viral clearance. Moreover, since viral detection assays were only performed in miniBAL samples and not in nasopharyngeal swabs, we formally cannot exclude that some patients, classified as virus negative in our study, actually did carry virus in the upper airways that later (after collection of the last miniBAL sample) could have resulted in a lower airway infection. Therefore, results should be interpreted with caution. Finally, on top of the forementioned restrictions, another significant limitation of our study is its insufficient power to properly correct for baseline differences, or to perform (propensity score) matched analyses. Larger studies are warranted and results from this study could be used for power calculations.

Notwithstanding the findings in our study, viral (re)activity is still likely to be one of the many factors contributing to the development of ARDS in patients following major surgery (9,11). It is known that major surgery, transfusions and certain comorbidities (diabetes mellitus, cardiovascular disease, obesity and old age) are associated with dysregulation of innate and adaptive immunity, including impaired antigen presenting capacity, impaired production of pro-inflammatory mediators, inhibition of T-cell activity, dysregulation of cytokine production, disruption of Th1 and Th17 pathways, and increased apoptosis of immune cells (15). This makes the body vulnerable to reactivation and a more severe clinical course of respiratory viruses (15). Subsequent disruption of the alveolar side of the endothelial/alveolar interface eventually leads to impaired gas exchange, disturbed P/F ratio, ARDS and in the end a protracted postoperative clinical course.

CONCLUSION

In this study we have demonstrated the presence of respiratory viruses in the perioperative phase in 12% of cardiac surgery patients that had no symptoms of a respiratory infection. Only low amounts of often low pathogenic viruses - as indicated by high CT-values – were detected and similarly present in and out of the ILI season. Although in our study the presence of respiratory viral genetic material was not associated with worse pulmonary outcomes after cardiac surgery, larger investigations are needed to determine the importance of the different types of respiratory viruses and the importance of viral load on the perioperative course after heart surgery.

LIST OF ABBREVIATIONS

aCCI:	adjusted Charlson Comorbidity Index
ARDS:	Acute Respiratory Distress Syndrome
CT-value:	Cycle Threshold value
CPB:	Cardio Pulmonary Bypass
CMV:	Cytomegalovirus
COPD:	Chronic Obstructive Pulmonary Disease
DNA:	Desoxyribo Nucleic Acid
hCoV:	human Corona Viridae
hMPV:	human Metapneumovirus
HSV:	Herpes Simplex Virus
ICU:	Intensive Care Unit
ILI:	Influenza Like Illnesses
IVA:	Influenza Virus Type A
IVB:	Influenza Virus type B
P/F ratio:	PaO2/FiO2 ratio
PCR:	Polymerase Chain Reaction
RSV:	Respiratory Syncytial Virus
RV:	Rhino Virus
RNA:	Ribo Nucleic Acid
SD:	Standard Deviation
SARS-CoV-2:	Severe Acute Respiratory Syndrome CoronaVirus-2

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SUPPLEMENTARY MATERIAL

Contains:

PART TWO

Predicting lung injury after cardiac surgery

4

Leukocyte and platelet activation in cardiac surgery patients with and without lung injury: A prospective cohort study

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https://www.ncbi.nlm.nih.gov/pmc/articles/PMC10203378/pdf/ivad062.pdf

ABSTRACT

Objections: Development of acute lung injury after cardiac surgery is associated with an unfavorable outcome. Acute respiratory distress syndrome in general is, besides cytokine- and interleukin activation, associated with activation of platelets, monocytes and neutrophils. In relation to pulmonary outcome after cardiac surgery, leukocyte- and platelet-activation is described in animal studies, only. Therefore, we explored the peri-operative time course of platelet- and leukocyte- activation in cardiac surgery and related these findings to acute lung injury assessed via PaO2/FiO2 (P/F) ratio measurements.

Methods: a prospective cohort study was performed, including 80 cardiac surgery patients. At five timepoints, blood samples were directly assessed by flowcytometry. For time course analyses in low (<200) versus high (>/=200) P/F ratio groups repeated measurements techniques with linear mixed models were used.

Results: Already before the start of surgery platelet activatability (p =0.003 for Thrombin Receptor Activator Peptide and p=0.017 for Adenosine Di Phosphate) was higher, and expression of neutrophil activation markers was lower (CD18/CD11; p = 0.001, CD62L; p = 0.013) in the low P/F group. After correction for these baseline differences, the peri-and postoperative Thrombin Receptor Activator Peptide induced thrombocyte activatability was decreased in the low P/F ratio group (p 0.008), and a changed pattern of neutrophil activation markers was observed.

Conclusion: Prior to surgery, an upregulated inflammatory state with higher platelet-activatability, and indications for higher neutrophil turnover was demonstrated in cardiac surgery patients that developed lung injury. It is difficult to distinguish whether these factors are mediators, or also etiologically related to development of lung injury after cardiac surgery and further research is warranted.

INTRODUCTION

Cardiac surgery induces a systemic inflammatory response syndrome with an incidence of 42%, that can lead to concurrent single- or multiple organ dysfunction (1). Development of acute lung injury and even acute respiratory distress syndrome (ARDS) in this respect is reported in 0.5-20% of patients after cardiac surgery (1-3), and is associated with a complicated postoperative course (1, 3, 4), high mortality (50-90%) (2, 4-7), and significant long term physical and psychological sequelae (7).

The pathophysiology of postoperative acute lung injury is thought to lie in inflammation-induced disruption and increased permeability of the alveolar-capillary membrane, and development of pulmonary oedema (8). Furthermore, it is thought to be evoked by multiple successive factors, such as pre-existent impaired left ventricular function, use and duration of cardiopulmonary bypass (CPB), lung ischemia-reperfusion injury, transfusion of blood products, and complexity of surgery (2,5). Complement activation through both the classical and alternate pathways, and subsequently or concurrently released proinflammatory cytokines (TNF-α, IL-1, IL-2, IL-6, IL-8) and anti-inflammatory cytokines (IL-10, IL-1ra, TNFsr1 and 2, and transforming growth factor) influence the magnitude and severity of the inflammatory response after cardiac surgery and development of ARDS (5,9). A prominent role in the development of ARDS in general has been assigned to activated platelets, monocytes and neutrophils (10,11). Studies in cardiac surgery demonstrated that platelet- and leukocyte activation, and platelet-leukocyte aggregation was increased (8,12,13), and suggested endothelial adhesion and entrapment of platelets and polymorphonuclear neutrophils in the lungs (5,14). However, these studies have described flow cytometric patterns of platelets and leukocytes after cardiac surgery in general, but relating them to postoperative development of acute lung injury and ARDS has only been done to a limited extend in combination with other biomarkers (15), or in animal studies (14,16).

It was our hypothesis that increased platelet and leukocyte activation patterns, as can be measured by flow cytometry, would be present in cardiac surgery patients that developed acute lung injury as compared to cardiac surgery patients that did not develop acute lung injury. Therefore, in this study we aimed to further explore the intra- and postoperative time course of platelet activation and activatability, leukocyte activation and the platelet-leukocyte interactions in adult cardiac surgery patients and relate these findings to the occurrence of acute lung injury. Further insight into platelet- and leukocyte associated biomarkers related to acute lung injury after cardiac surgery is important for understanding the pathophysiology and to bring personalized preventive measures a step closer to clinical practice.

PATIENT POPULATION AND METHODS

Ethical statement

The study was approved by the Medical Ethical Committee (27-09-2011; protocol P11-117) of the Leiden University Medical Center and was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments (17). The trial was registered at the International Clinical Trials Registry Platform (NTR 5314, 26-05-2015) The Strobe statement checklist (18) is available in supplement Chapter 4.1. Written informed consent was obtained from all included patients on the day before surgery.

Study design and study population

A prospective cohort study was performed in of the ICU of a tertiary referral hospital, the Leiden University Medical Center in the Netherlands. Eligible patients were adults undergoing elective cardiac surgery. A dedicated researcher and laboratory technician were available to ensure immediate transportation of samples to the laboratory and direct analyses on a readily available flow cytometer in the laboratory on all consecutive study days. Therefore, patients could only be considered for inclusion, when surgery was planned early morning on a Monday or Tuesday. The maximal capacity was limited to one patient a week, and only when critical laboratory and research personnel were available. The selection of Monday or Tuesday did not introduce a bias of specific types of surgery as in our hospital all types of cardiac surgery are performed equally over weekdays. The exclusion criteria were inability to sign informed consent, being less than 18 years old, emergency operations, participation in another study, and pre-operative use of corticosteroids. The Follow up period was until discharge form the hospital.

Sample Size:
A formal sample size estimation was not possible because only a few earlier studies were available (12,19-23), and these studies were small (all less than 20 patients), research questions were various, and tests for platelet- or white blood cell activity were different from the methods we intended to use. Therefore, we applied a pragmatic approach to include as much patients as possible in the years that were assigned to conduct this study.

Perioperative care

All patients visited the pre-operative outpatient clinic for pre-operative screening. All patients were admitted one day before surgery. Perioperative care for cardiac surgery patients is standardized in the Leiden University Medical Center and follows a pre-established care path. All details regarding the peri-operative care are summarized in supplement Chapter 4.2.

Data collection

All pre-, intra- and postoperative data and clinical parameters were obtained from the electronic patient database (EPD) system of the hospital. This EPD is used preoperatively, in the operating room and in the ICU. In the ICU, continuous hemodynamic and ventilation monitoring is recorded. Four times per day an arterial blood gas analysis is done and more frequently on indication.

Sample collection

At five different time points blood samples were drawn (before the start of anesthesia/ surgery at the holding facility (T0), 1 hour after weaning of CPB (T1), T1+ 3 hours (T2), first postoperative day (T3), second postoperative day (T4). Samples were collected in lithium heparin vacuettes. Samples were transported to the laboratory and analyzed immediately after collection.

Flow cytometry analyses

The Beckman Coulter FC500MPL was used for flow cytometric analyses. A designated laboratory employee was available for our study. Before starting the actual measurements of our study samples, daily quality checks were performed to ensure the reliability and accuracy of the results. For platelet activation markers and leukocyte-platelet complexes, dilutions were created using 3% Bovine serum albumin (BSA)/ PBS).

Platelet activation: Fluorescein antibodies against CD61 (FITC) and CD62 (PE) were used as platelet activation markers. Adenosine diphosphate (ADP) and thrombin receptor activator for peptide (TRAP) were chosen as well-known examples respectively weak and strong platelet agonists, that in our hands showed good day to day reproducibility of agonist-concentration dependent aggregation responses. Analyses were done with eight increasing concentrations of the different agonists.

Leukocyte activation: White blood cells were adjusted to a maximum concentration of 20 x 106/ml in phosphate buffered saline (PBS), Fluorescein antibodies against Neutrophil CD66b (FITC) and activation markers CD11b/18 (PE), CD62L (PE) were used, Optilyse-C for lysis of the erythrocytes and Stem Count beads for calibration

Leukocyte-Platelet complex formation: White blood cells were adjusted to a maximum concentration of 20 x 106/ml in phosphate buffered saline (PBS). Fluorescein antibodies against monocyte CD14 (FITC), Neutrophil CD66b (FITC) and platelets CD42b(PE), for calibration beads Stem Count were used. Optilyse-C for lysis of the erythrocytes and Stem Count beads for calibration.

The above laboratory studies yielded dose response activation curves, from which the maximum, the minimum and the mean percentage, which is an equivalent of the area under the activation curve (24), of responsive cells can be derived. Since the mean percentage of responsiveness is an aggregate measure, reflecting both the maximum number of responsiveness and the minimum agonist concentration at which cells respond (24), it was decided to use the mean percentage. Results were expressed as mean percentage responsive cells in the dose-response activation curve.

Endpoints

The primary aims of this study were monitoring of the perioperative time course of leukocytes, platelets and their activation status and complex formation in adult cardiac surgery patients with and without acute lung injury. As a measure of lung injury, we used the PaO2/FiO2 ratio (P/F ratio). This is an objective tool to identify acute hypoxemic respiratory failure when supplemental oxygen and positive pressure ventilation is being administered, and, as such, a measure of acute lung injury and an important item in the definition of ARDS according to Berlin Criteria (25).

In thoracic surgery patients, a P/F ratio < 200 is associated with unfavorable outcome, and each increase in ARDS severity category, which is per definition only determined by a decline of the P/F ratio, is reported with a significant increase in morbidity and mortality (4). Hence, to distinguish mild from more severe and clinically more relevant lung injury patients, we dichotomized the P/F ratio in two subgroups of lung injury: moderate to severe impaired respiratory state (lowest postoperative P/F ratio < 200 mmHg) versus mildly impaired and normal respiratory state (lowest postoperative P/F ratio ≥ 200 mmHg).

To determine surgery-related lung injury, we used, analogous to these Berlin criteria, a maximum time period of 1 week after surgery. Although FiO_2 is notoriously unreliable once at low-flow oxygen supply (27,28), and PaO_2 is only measured on clinical indication at the general thoracic surgery ward and hence the P/F ratio was not available in extubated patients, it is unlikely that we missed patients who developed a low P/F ratio and lung injury on the ward, especially as all patients are transferred back to the ICU once (non-) invasive ventilation is mandatory.

Statistical analyses

in consultation with the Departments of Biomedical Data Sciences and Clinical Epidemiology of the Leiden University Medical Centre, a statistical analysis plan was defined in advance, before disclosure of the data. The statistical analysis was carried out according to plan.

Demographic and clinical characteristics are presented as mean (SD) or median (IQR) for continuous variables and as absolute values and percentages for categorical variables. For the normal/mildly impaired versus moderate/severe respiratory groups baseline characteristics were compared with a Fischer's exact test for categorical variables and Mann-Whitney U test for continuous variables.

Furthermore, the perioperative time course of white blood cells, platelets, platelet- and leukocyte activation, and platelet-leukocyte complex formation was tested for significance. Baseline differences in P/F ratio groups were tested with a T-test, and the further perioperative time course was tested with repeated measurements techniques, i.e. Linear Mixed Model. Of particular interest was the (a) effect of P/F ratio groups, i.e. high (>200) versus low (<200) P/F ratio and (b) effect of the time in the different P/F ratio groups. To distinguish baseline differences (i.e. at T0) from postoperative effects, the baseline value was compared in both P/F ratio groups, using T-test, and introduced as a variable in the repeated measurement analysis.

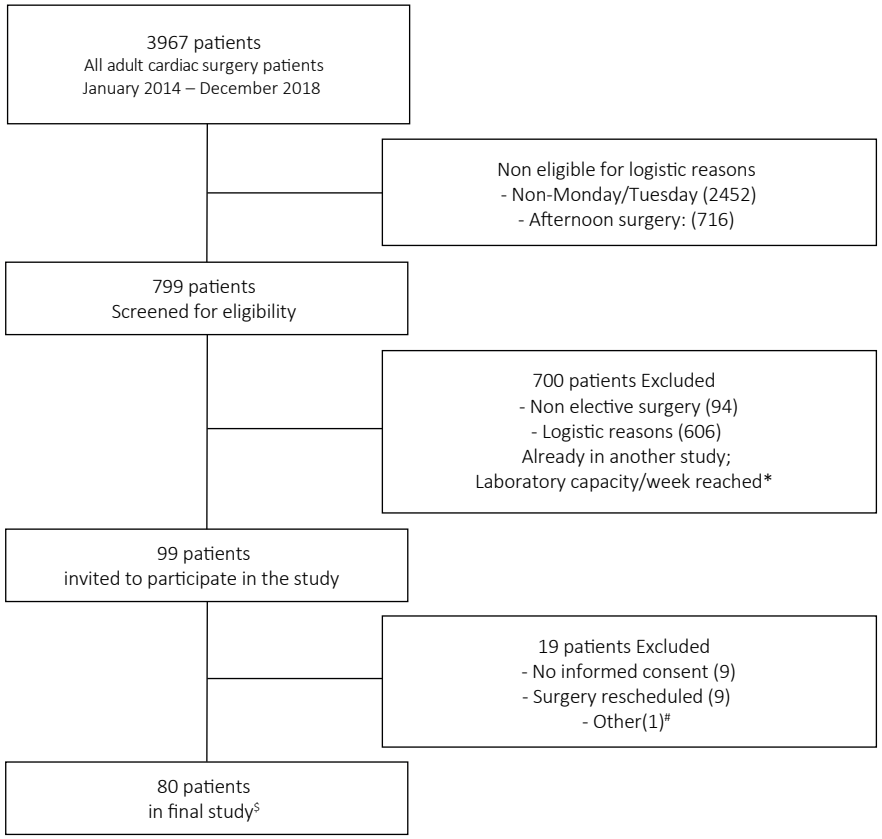
The statistical analyses were conducted using the SPSS (Statistical Package for the Social Sciences), release 25.0 (SPSS Inc., Chicago)

RESULTS

A total of 80 patients was included in this study from January 2014 to January 2018. In Figure 1 a flow chart of the inclusion process is shown. Four patients were excluded from the analyses, since they were already extubated when they arrived at the ICU, and no P/F ratios could be derived.

The patient characteristics are summarized in *Table 1*. Patients were predominantly middle-aged male, BMI was higher in the low versus high P/F ratio groups, left ventricular function was worse in the low compared to the high P/F ratio group, Euro-score was higher in the low P/F ratio group, and heart failure surgery was performed more frequently in the low P/F ratio group.

Figure 1. Flowchart inclusion



** A precise logistic network, consisting of a dedicated researcher and laboratory employee, was set up to ensure immediate transportation to the laboratory and direct analyses of blood samples on a readily available flow cytometer in the laboratory on all consecutive study days. Therefore, patients could only be considered for inclusion when surgery was planned early morning on a Monday or Tuesday. And maximal capacity was limited to one patient a week.*

 # IV access difficulties (history of chemotherapy) prohibited drawing blood form a peripheral vein. pre-operative blood sampling would mean additional invasiveness and was judged too burdensome for the patient.

$ 4 patients were excluded from analyses, since they were extubated at the operation room and no postoperative P/F ratio was available.

Table 1. Patient Characteristics

Characteristics		pF-ratio < 200 n = 23		pF-ratio ≥ 200 n = 53		p value*
Demographic parameters						
Age (yr) (mean, SEM)		66.6	2.1	65.0	1.2	0.583
Gender (male) (n, %)		12	55	26	49	0.147
BMI (kg/m^2) (mean, SEM)		25.8	1.9	21.7	0.7	0.217
Co-morbidity (n, %)						
Myocardial infarction in history		4	18	7	13	0.032
PCI in history		4	18	1	2	0.021
Thoracic surgery in history		0	0	2	4	0.491
Hypertension		9	41	14	26	0.147
Malignancy		0	0	1	2	0.707
Chronic kidney insufficiency		1	5	0	0	0.293
Chronic Liver disease		2	9	0	0	0.083
Diabetes		4	18	0	0	0.006
COPD		1	5	8	15	0.478
Smoking		12	55	21	40	0.205
Packyears (median, IQR)		12	50	20	25	0.493
Forced Vital Capacity (L) (median, IQR)		3.4	1.8	3.8	1.5	0.516
FEV1/VC (median, IQR)		69.5	8.6	74.4	9.6	0.328
Preoperative medication use (n, %)						
Diuretics		11	50	20	38	0,284
ACE blockers		13	56	25	47	0.195
Betablockers		15	65	29	55	0.276
Calcium antagonist		6	26	8	15	0.205
Statins		13	56	25	47	0.309
Thrombocyte aggretation blockers$		10	43	20	38	0.412
Ante-Surgery performance state (n, %)						
ASA	I	0	0	0	0	0.203
	II	4	18	5	9	
	III	16	73	41	77	
	IV	1	5	0	0	
LVEF	Good LVEF > 55%	9	41	23	43	0.048
	Reasonable LVEF 40-55 %	6	27	11	21	
	Moderate LVEF 25-40%	5	23	2	4	
	Poor LVEF < 25%	2	9	2	4	
	EuroSCORE 2 (logistic) (median, IQR)	3.0	7.7	2.2	2.3	0.014

Table 1. Continued

Characteristics	pF-ratio < 200 n = 23		pF-ratio ≥ 200 n = 53		p value*
Surgical parameters					
Surgical procedure (n, %)					
CABG	3	14	11	21	0.415
CABG + single valve	4	18	6	11	
CABG + multiple valve	1	5	1	2	
Single valve	5	23	13	25	
Multiple valve	1	5	6	11	
Thoracic Aorta surgery (±Valve/CABG)	4	18	13	25	
Heart failure surgery	3	14	4	8	
Other	2	9	4	8	
Surgical duration					
Surgery (min) (median, IQR)	360	126	345	90	0.361
Cardiopulmonary bypass (min) (median, IQR)	115	106	110	71	0.704
Corticosteroid therapy #					
Intraoperative use, overall (n,%)	8	36	11	21	0.450
Dexamethason 0,1-0,5 mg/kg	6	27	8	15	
Hydrocortison 100 mg	2	9	2	4	
Prednisolon 0,5 mg/kg	0	0	1	2	
ICU admittance risk Score					
APACHE IV (medIan, IQR)	58	17.7	44	19	0.012

** Chi square for categorial parameters. Means and T-test for normally distributed parameters. Median and Mann Withney for skewed distributed parameters.*

$ Antiplatelet therapy is discontinued 5-10 days before surgery, unless patients underwent recent coronary artery stenting or suffer from instable angina pectoris and had a semi-acute indication for surgery.

 # Patients that had an indication for corticosteroids pre-operatively were excluded from this study. Indications for intraoperative corticosteroid use are systemic inflammatory response with high vasopressor demand, allergic reaction and bronchospasm.

Abbreviations: APACHE Acute Physiology and Chronic Health Evaluation; ASA American Standards Association; BMI Body Mass Index; IQR Inter Quartile Range; FEV1 Forced Expiratory Volume in 1 second; LVEF Left Ventricular Ejection Fraction; PCI Percutaneous Coronary Intervention; SEM Standard Error of Means; VC Vital Capacity

Development of ARDS was more common in the low (P/F < 200) versus the high (P/F >/= 200) P/F ratio group (14/23 (64%) versus 9/53 (17%) (p<0.001). Furthermore, patients in the low P/F ratio group spent a longer time on the mechanical ventilator, had a longer ICU stay, and died more often *(Table 2)*.

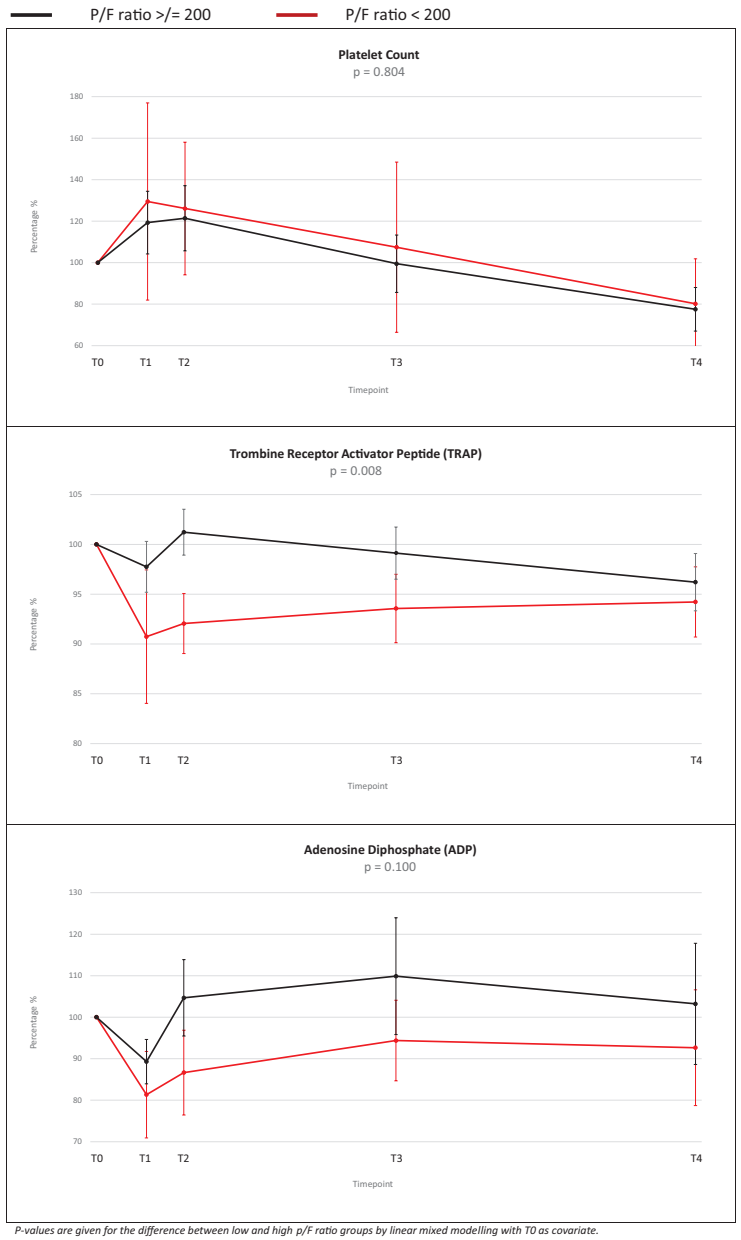
Table 2. Patient Outcome

Characteristics	pF-ratio < 200 n=23		pF-ratio ≥ 200 n=53		p value*
Outcome					
Ventilation time, minutes (median, IQR)	840.0	2704	675.0	405	0.005
Length of Stay ICU, hours (median, IQR)	43.5	161	22.5	3	0.047
Length of Stay hospital, days (median, IQR)	11.0	18	8.0	4	0.064
ARDS within 7 days after surgery (n, %)	14	64	9	17	< 0.001
Mortality (n, %)	2	9	0	0	0,088

**Chi square for categorial parameters. Means and T-test for normally distributed parameters. Median and Mann Withney for skewed distributed parameters. Abbreviations: ARDS Acute Respiratory Distress Syndrome; ICU Intensive Care Unit; IQR Inter Quartile Range*

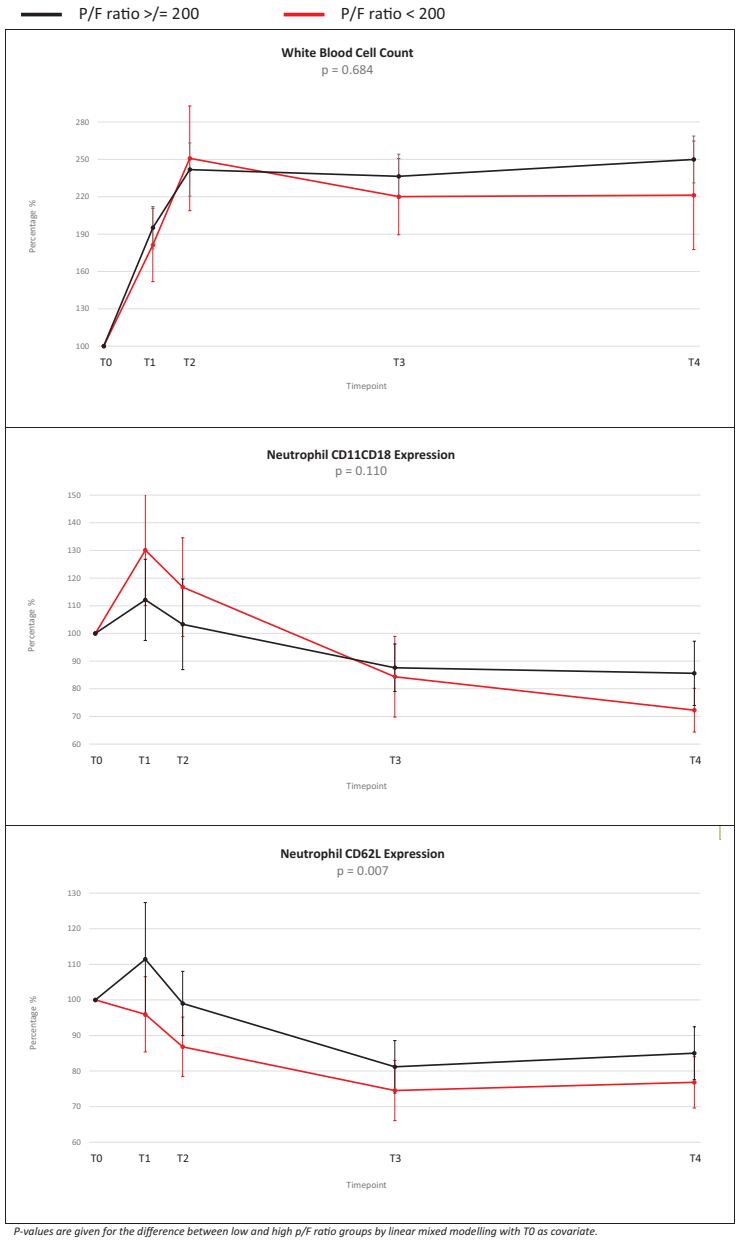
Already before the start of surgery (*Figure 2*), platelet activatability, as measured after adding ADP and TRAP, was higher in the low compared to the high P/F ratio group (p=0.003 for TRAP and p=0.017 for ADP). Expression of neutrophil activation markers was lower for both CD18/CD11 (p=0.001) and CD62L (p<0.013) in the low compared to the high P/F group. The perioperative time course of platelets, platelet activatability, WBC and neutrophil activation markers and leukocyte-platelet complex formation are shown in figures 2-4. To visually correct for the T0 differences, T0 is preset for both groups at 100% and the subsequent values are then shown in relation to this T0. In the time course analyses, only TRAP induced thrombocyte activatability decreased more in the low P/F ratio group (p=0.029). Additionally, a clear neutrophil activation pattern was observed in the latter group with increasing CD11CD18 and lowering of CD62L.

Figure 2. Platelets and platelet activatability in time for low (<200) and high (>/= 200) P/F ratio (adjusted for T0 difference)



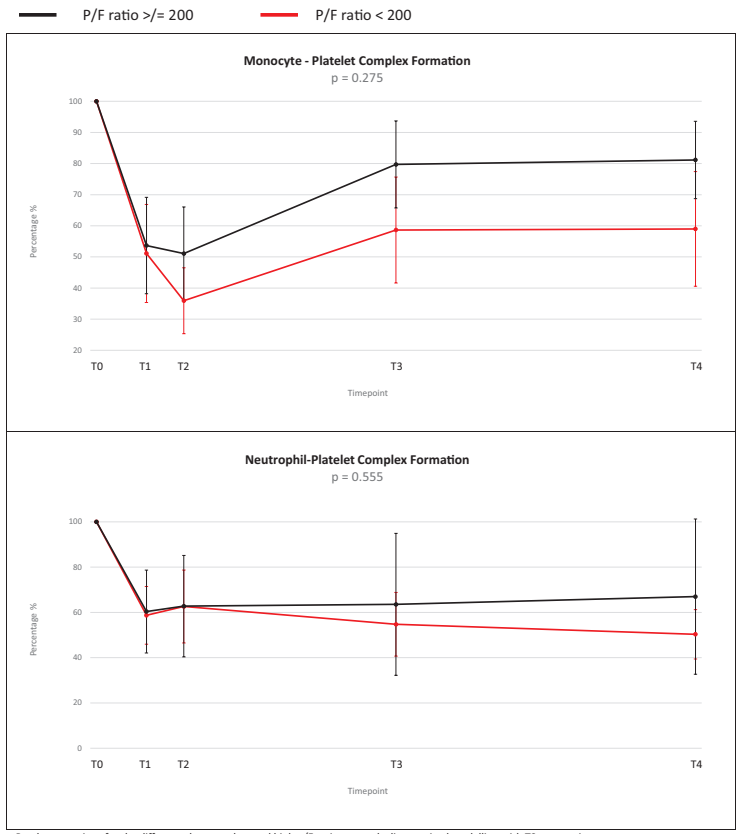
P-values are given for the difference between low and high p/F ratio groups by linear mixed modelling with T0 as covariate.
T0 = Pre-operative, T1 = End CPB + 1 hour, T2 = T1 + 3 hours, T3 = T1 + 18 hours, T4 = 2nd post operative day.

Figure 3. Leukocytes and Leukocyte activation in time for low (<200) and high P/F ratio (adjusted for T0 difference)



P-values are given for the difference between low and high p/F ratio groups by linear mixed modelling with T0 as covariate.
T0 = Pre-operative, T1 = End CPB + 1 hour, T2 = T1 + 3 hours, T3 = T1 + 18 hours, T4 = 2nd post operative day.

Figure 4. Complex formation in time for low (<200) versus high (>/= 200) P/F ratio (adjusted for T0 difference)



P-values are given for the difference between low and high p/F ratio groups by linear mixed modelling with T0 as covariate.
T0 = Pre-operative, T1 = End CPB + 1 hour, T2 = T1 + 3 hours, T3 = T1 + 18 hours, T4 = 2nd post operative day.

DISCUSSION

In this prospective cohort, we measured platelet and leukocyte associated biomarkers in the pre-, intra- and postoperative phase of 80 cardiac surgery patients and we related these biomarkers to the development of acute lung injury, defined by P/F ratio. We demonstrated that already before the start of surgery, a significantly higher capacity of platelets to be activated by ADP and TRAP was present in the patient group that developed acute lung injury as defined by P/F ratio < 200. Furthermore, lower expression of both CD11/CD18 and CD62L was present in the low P/F ratio group. After

correction of T0 differences, the post-operative course for the low P/F ratio group showed a significant decrease in the TRAP-induced platelet reactivity, and a typical pattern of the 2 neutrophil-activation markers: though not significantly different, neutrophil CD11/CD18 and CD62L expression showed an inversed change, i.e. increasing CD11/CD18 expression and decreasing CD62L expression, that could be interpreted as a higher turn-over of granulocytes in the lower P/F ratio group (29,30).
Some limitations of our study must be addressed, the most important of which is the sample size. Although, to our knowledge, this is the largest study of platelet and neutrophil activation in cardiac surgery patients and the first to relate this data to an acute lung reaction after cardiac surgery, still the sample size, based on pragmatic grounds, is not large enough to allow correction for- or in depth subgroup analyses of- the different types of surgery, steroid use, antiplatelet therapy, and other factors that might confound the outcomes in this study.

Secondly, the pre-operative flow cytometric differences in the two P/F ratio groups are not necessarily etiological factors in the development of pulmonary complications. They could also be mediators associated with other pre-operatively present co-morbidity, such as impaired left ventricular function, previous coronary artery disease, chronic heart failure, arteriosclerosis and atrial fibrillation; all well known risk factors itself for development of acute lung injury and unfavorable outcome after cardiac surgery (26), and all associated with increased inflammatory status, platelet activation, and endothelial driven hypercoagulability (27-30). In our study, these risk factors were more common in patients in the low P/F group and therefore, a (partly) non-causal or indirect relation of our biomarkers with this outcome cannot be excluded.

Furthermore, the local role of platelets and neutrophils (and their complexes) in the lung itself can not automatically be translated from measurements in circulating blood. In this respect it would have been interesting to study platelet and neutrophil activation status in blood and alveolar fluid concomitantly. Likewise, to clarify the role of the CPB, sequential sampling of blood in both inlet and outlet of CPB would have been informative.

Notwithstanding all the aforementioned limitations, clear differences in platelet activatability and neutrophil counts were demonstrated at baseline. Although, the low P/F group showed a more typical neutrophil activation pattern in the post-surgery time course with clear inverse reacting CD11CD18 (up) and CD62L (down), other parameters, when percentualized on baseline level, showed mostly not significant but discrete time courses after surgery in both outcome groups. From this it must be realized that differences in absolute values during the first postoperative days remain.

Although a non-etiological effect between flowcytometric results and outcome is uncertain and extrapolating whole blood results to pathophysiological processes in the lungs has important limitations, the results of our study cannot entirely be brushed off. Our findings suggest that preoperative presence of increased activatability of platelets, lower neutrophil activation marker expression (with possibly also a higher neutrophil turn-over) may have an etiologic role in the development of lung injury after heart surgery. The differences in biomarker baseline values between both study P/F ratio groups fit with the well-known pathophysiology of acute lung injury in other patients' groups. In previous studies in patients with ARDS, neutrophils indeed are shown to migrate to the inflamed tissue site, where a multistep of neutrophil-endothelial tethering, rolling, adhesion, crawling and transmigration takes place. Subsequently, neutrophil extracellular traps enable them to elicit their immunological action locally (4,31,32). In accordance, also earlier observational studies in cardiac surgery reported on activation of monocytes, neutrophils and platelets (12,13,16,20,33), endothelial transmigration and influx of these complexes in several organ systems as well (14,16,33). Such inflammatory processes could well be augmented if pre-surgery platelets and neutrophils are already activated as was the case in our low P/F group.

Despite the limitations of this study, especially the limited sample size, there are some important additional research questions that arise when considering the signals that emerged from this study. At first, it would be worthwhile to further explore thrombocyte and neutrophil activation patterns in predefined subgroups, such as the various subtypes of surgery, and in patients with and without antiplatelet therapy. Also, it would be interesting to further explore the activation patterns in patients that, according to prevailing practice, did receive postoperatively steroids versus those who did not. Furthermore, in this study, due to sample size, we were unable to do reliable prognostic studies and we refrained from making ROC curves and calculating sensitivity and specificity. However, in future studies, it would be valuable to explore prediction capacity of flow cytometric markers in addition to already known and applied prediction models. Finally, the peri-operative signals of thrombocyte and neutrophil activation in this specific study population of thoracic surgery patients and the association with pulmonary outcomes, might also be extended to other patient groups. It is well conceivable that similar processes play a role in other major surgery types, such as vascular/aortic surgery, large gastrointestinal surgery, and transplant surgery. Future studies are necessary to elucidate inflammatory responses and specifically the role of platelet and white cell activation after other types of major surgery.

CONCLUSION

We showed, that prior to commencement of cardiac surgery an upregulated inflammatory state is present in patients who develop (acute) lung injury. The observed higher platelet-activatability and signs of a higher neutrophil turn over before surgery, and a typical neutrophil activation pattern later could well contribute to more severe acute lung injury in this respect. Further research, however, is needed and should at least involve (a) predefined subgroups (various surgery types, steroid use, antiplatelet therapy) (b) the role of the CPB circuit on platelets and neutrophils, (c) the influence of transfused blood products and (d) the neutrophil-platelet interactions at the alveolar-capillary lung level itself. If eventually, biomarkers on platelets and neutrophils, and lung vasculature interactions can be causally linked to acute lung injury, it would bring personalized medicine with patient-specific considerations with regard to interventions to be performed, expected outcomes, and specific preventive measures a step closer.

LIST OF ABBREVIATIONS

ADP:	Adenosine Di Phosphate
APACHE:	Acute Physiology and Chronic Health Evaluation
ARDS:	Acute Respiratory Distress Syndrome
ASA:	American Standards Association
BMI:	Body Mass Index
CABG:	Coronary Artery Bypass Grafting
CPB:	Cardio Pulmonary Bypass
EPD:	Electronic Patient Dossier
ICU:	Intensive Care Unit
IL:	Interleukin
IQR:	Inter Quartile Range
FEV1:	Forced Expiratory Volume as measured in 1 second
LVEF:	Left Ventricular Ejection Fraction
P/F ratio:	PaO2/FiO2 Ratio
PCI:	Percutaneous Coronary Intervention
SEM:	Standard Error of Means
TNF:	Tumor Necrosis Factor
TRAP:	Trombine Receptor Activator Peptide
VC:	Vital Capacity

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SUPPLEMETARY MATERIAL

Contains:

4.1 Strobe statement

4.2 Perioperative management in detail

5

Perioperative proadrenomedullin adds to EuroSCORE to predict ARDS and clinical outcome in cardiac surgery ICU patients: a prospective cohort study

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N.P. Juffermans, R.B.P. de Wilde, T. Stijnen, E. de Jonge, R.J.M. Klautz, M.S. Arbous.

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ABSTRACT

Introduction: Acute respiratory distress syndrome (ARDS) after cardiac surgery is associated with unfavorable outcome and its prediction is important. Single-value proadrenomedullin (proADM) has been identified as a prognostic biomarker in various clinical situations and shows to increase predictive capacity of established clinical scoring systems. However, pre-operative levels do not include the impact of surgery itself on inflammatory response and development of ARDS. Therefore, we assessed the predictive value of the pre-to-postoperative proADM increase, in addition to EuroSCORE, for ARDS and clinical outcomes in intensive care patients after cardiac surgery.

Methods: We performed a prospective cohort study at a tertiary university ICU. The study population comprised of adult patients undergoing elective cardiac surgery. Perioperative care was standardized. ProADM was measured in sequential venous blood samples. Clinical data were derived from electronic patient records. Linear mixed model and logistic regression techniques were used for data analysis.

Results: The absolute pre- to postoperative proADM increase was associated with occurrence of ARDS (OR 3.55, 95% CI 1.17-10.84), and length of stay (LOS) on the ICU > 48h (OR 30.03, 95 % CI 11.73-48.34). A perioperative proADM-change > 1.5 nmol/L could predict ARDS after cardiac surgery (specificity 81%, sensitivity 62%). Adding perioperative proADM-change to EuroSCORE improved the latter prediction for ARDS and LOS-ICU, while as isolated predictor, proADM change was superior to EuroSCORE.

Conclusion: Perioperative proADM change increases the predictive value of the EuroSCORE for ARDS and identifies patients at risk for development of ARDS and increased LOS-ICU. This finding will enable clinicians to focus and direct their lung-protective interventions to minimize or prevent secondary pulmonary injury in high risk patients at the intensive care unit (ICU) after cardiac surgery and tailor postoperative care to those who need it most.

INTRODUCTION

The incidence of the systemic inflammatory response syndrome (SIRS) after cardiac surgery with cardiopulmonary bypass (CPB) is 42 percent (1). SIRS can lead to organ dysfunction (2,3), in which lung involvement, from mild to severe acute respiratory distress syndrome (ARDS), is one of the most feared complications. Notwithstanding its rarity after cardiac surgery, i.e. 0.5-2%, severe ARDS is associated with a complicated post-operative course and high mortality (50-90%) (4, 5). To initiate appropriate preventive measures, such as lung protective ventilation, oxygen reduction, adequate fluid management (6-8), prediction of ARDS at an early postoperative stage would be of great importance.

Cardiac risk assessment models, such as the EuroSCORE (9), are useful for quality control and provide guidance in clinical management, as they are associated with a complicated post-operative course and mortality (10]. The EuroSCORE comprises 17 items: general patient information, cardiac status and type of operation, but it does not include a specific biomarker that reflects the magnitude of the perioperative systemic inflammatory response, which often is the pathophysiological pathway to multi-organ failure and sometimes to death. In this respect improvement of the predictive value of EuroSCORE was already demonstrated by the use of a single preoperative measurement of proadrenomedullin (proADM), the biologically more stable prohormone of adrenomedullin (ADM) (11), a multi-potent and biologically active peptide affecting vasomotor status (12-14) as well as being a marker of inflammation (15).

We hypothesized that a pre-to-post surgical change in proADM, including both the individual pre-operative inflammatory state, and the inflammatory response to the cardiac surgical procedure, might in this respect be more meaningful than a single measurement. Furthermore, proADM is known to be expressed in many pulmonary cell types that mediate local vasoactive effects (16), and is likely to be of value in predicting ARDS.

In the present study we therefore studied: (1) the time-course of proADM following cardiac surgery, and (2) the predictive value of the perioperative change of proADM for the development of ARDS and other relevant clinical outcomes in cardiac surgery patients. Finally, we studied (3) the additive value of the perioperative change of proADM to the EuroSCORE in predicting ARDS.

METHODS

Study design and study population

A prospective cohort study was performed in a 26 bed ICU of a tertiary referral hospital. The study was approved by the Medical Ethical Committee (protocol P117-11) and conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments (17).

Eligible patients were adults undergoing elective cardiac surgery. The exclusion criteria were inability to sign informed consent, less than 18 years old, emergency operations, and participation in another study. Written informed consent was obtained from all included patients the day before surgery.

Anesthesia and Surgery

Anesthesia was standardized and consisted of premedication with lorazepam, induction with propofol, remifentanil and rocuronium. Anesthesia was maintained with midazolam, propofol and remifentanil or sufentanil continuously. Before start of surgery all patients received a central venous and an arterial catheter, standard surgical antibiotic prophylaxis, and tranexamic acid to minimize perioperative blood loss. After venous and arterial cannulation, bypass was commenced using a heart-lung machine (S3; Sorin Group) with a centrifugal blood pump (Revolution; Sorin Group) with rates set at 2.0 to 2.6 l/m2/min and a hollow fibre membrane oxygenator (MaquetQuadrox-I or Terumo FX15). The cardiopulmonary bypass (CPB) circuit was primed with hydroxyethyl starch 130 (Voluven 6%; Fresenius Kabi Norge AS), Mannitol 15%, Ringer's solution and Heparine. Tubing was coated with bio-inert heparin-free polymers (SAFELINE; MAQUET Holding BV & Co KG). Intermittent warm antegrade blood cardioplegia was instituted every 15 minutes for a period of two minutes. Heparin was administered before the start of CPB as a 300 International Units/kg bolus and subsequently adapted to reach an activated clotting time > 400s. During CPB patients were ventilated according to standard protocol with low pressure and low tidal volume to prevent atelectasis of the lung, except for procedures in which persistent ventilation obstructed surgical procedures. During CPB, core temperature was maintained at 34oC to 36oC. Active cooling was solely used during aortic surgery to prevent brain ischemia. At the end of CPB, heparin was antagonized with protamine sulphate, titrated 1:1 to the initial dose of heparin. Inotropic and vasoactive agents were administered on indication. Duration of anaesthesia, surgery, CPB and aorta clamping were recorded. Applied transfusion triggers were congruent with the national guidelines (18) and corticosteroids were only administered in pre-defined situations.

Postoperative care

Patients were treated according to a "fast-track" protocol (19). When hemodynamically stable and with a core temperature of> 36 °C, patients were extubated when the following criteria were met: 1) Ramsay score 2-3, 2) Arterial pO2 > 9 kPa and FiO2 40%, 3) pH > 7.30 and 4) decreasing thoracic drain production < 50-100ml/hour. After extubation, sufentanil was replaced by methadone. Patients were discharged from the ICU on the first post-operative day, unless the patient's hemodynamic and respiratory status required a prolonged ICU stay or sometimes in case of unavailability of beds at the thoracic surgery ward. Hemodynamic monitoring was performed by an arterial and central venous catheter and, on indication, with a pulmonary artery catheter. In patients with low cardiac output, dobutamin, enoximone/milrinone, and noradrenaline were administered as inotropic and vasoactive agents.

Data collection

All pre-, intra- and postoperative demographic data and clinical parameters were obtained from the electronic patient database (EPD) system of the hospital (i.e. PDMS, Chipsoft and EPD vision). This EPD is used preoperatively, in the operating room and in the ICU. In all patients a EuroSCORE (9) was calculated and the NYHA-classification (20) and CCS-classification (21) were recorded. Mortality data were derived from the Dutch basic population registry in those cases in which the patient did not die in hospital but after discharge.

Pre- and postoperative chest X-rays were routinely performed and were judged by an independent radiologist, unaware of the proADM results from the blood samples. From day 1 postoperatively and further on, chest radiography was performed on indication.

Sample collection:
At nine different time points 5 ml blood samples were drawn (T0 = pre-surgery, T1 = after induction of anesthesia, T2 = 1 hour after weaning from CPB, T3 = at ICU arrival, T4 = 3 hours after ICU arrival, T5 = 6 hours after ICU arrival, T6 = 18 hours after ICU arrival, T7 = day 3 post-surgery and T8 = day 5 post-surgery), in Vacuette lithium-heparin collection tubes. Blood was sent to the laboratory immediately, processed (centrifuged for 5 min at 2500 g) and plasma was stored at minus 80 degrees Celsius.

After finishing sample collection of the included 40 patients, proADM levels in plasma were assessed using one reagent batch (in order to prevent lot-to-lot variation), according to the instructions of the manufacturer. Analysis of proADM was done on coded samples without knowledge of clinical data. The Thermo Fisher / BRAHMS KRYPTOR® Time Resolved Amplified Cryptate Emission (TRACE) technology (22) was

used to assess the levels of proADM. This pro-hormone of adrenomedullin is more stable and is known to be a reliable substitute of adrenomedullin (23).

Endpoints

The primary endpoint of this study was the development of ARDS according to Berlin criteria (24) within 7 days after surgery. Other causes of bilateral infiltrates were taken into account by the use of an end-of-surgical-procedure cardiac ultrasound to assess cardiac function and per-procedure ultrafiltration was also recorded to assess fluid overload. We dichotomized the Berlin severity score of ARDS in two subgroups of lung injury: severe and moderate ARDS (impaired PaO2/FiO2 <200 mmHg) versus mildly impaired and normal respiratory state (PaO2/FiO2 ≥ 200 mmHg).

Secondary outcomes were length of stay at the ICU (LOS-ICU), and LOS-hospital. We choose to dichotomize LOS-ICU and LOS-hospital into clinically relevant increased LOS-ICU and LOS-hospital for the cardiac surgical population. Before start of analyzing, a group of clinical experts were questioned on events thought to be relevant in the postoperative course of cardiac surgery patients. Following consultation of this panel of clinical experts, LOS ICU > 48 hours (this would cover some unnecessary stay due to shortage of beds at the ward) and LOS hospital > 7 days were selected as clinically relevant outcomes.

Statistical analyses

A statistical analysis plan was defined in advance, before disclosure of the data. The statistical analysis was carried out according to plan.

Firstly, we used descriptive statistics to assess mean levels of proADM on individual time points in the whole group and stratified for development of ARDS. The time course of proADM, corrected for baseline proADM, was tested with a repeated measurement technique (linear mixed model analyses, LMM) for significance.

Secondly, we assessed the predictive value of the EuroSCORE, and of perioperative proADM-change separately in a univariable model for prediction of ARDS, LOS-ICU > 48h and LOS-ICU > 7d. Since, in clinical practice, it is feasible to obtain blood samples directly after induction (T1) of anesthesia and at ICU admission (T3), it was planned to test this T1-T3 ProADM-change for its predictive value on clinical outcomes.

Thirdly, we assessed the additional value of T1-T3 proADM-change to the EuroSCORE by the use of a multivariable logistic regression model for the clinical outcomes under study. With the predicted values of the model with solely EuroSCORE and the model of EuroSCORE with T1-T3 proADM-change we did a ROC analysis and calculated a

c-statistic (Area under the Curve - AUC). We compared the C-statistic of the model with solely EuroSCORE with the c-statistic of the model with EuroSCORE and T1-T3 proADM-change. We tested the regression coefficient of T1-T3 proADM-change in the logistic model for significance. We considered the change of C-statistic (with and without proADM-change) to be significant if this p-value was < 0.05. Finally, we calculated for different cut-off points of T1-T3 proADM-change sensitivity and specificity for predicting ARDS.

The statistical analyses were conducted using the SPSS (Statistical Package for the Social Sciences), release 20.0 (SPSS Inc., Chicago)

RESULTS

From November 2011 to December 2012, 40 of 44 consecutive patients agreed to participate in the study. There were no patients lost to follow up and all patients were included in the analyses. The patient characteristics are shown in table 1. The patient population consisted of predominantly male (65%) patients, undergoing a spectrum of cardiac surgical procedures, i.e. coronary artery bypass grafting, valve surgery or heart failure surgery.

Table 1. Patients Characteristics

Characteristics	N=40 patients	
Demographic parameters		
age (yr) (mean, SE)	65.6	(2.3)
gender (male) (n, %)	25	(62.5 %)
BMI (kg/m^2) (mean, SE)	26.9	(0.57)
Co-morbidity (n, %)		
Myocardial infarction in history	7	(17.5 %)
PCI in history	10	(25 %)
Thoracic surgery in history	3	(7.5%)
Hypertension	22	(55 %)
Malignancy	4	(10 %)
Chronic kidney insufficiency	4	(10 %)
Diabetes	8	(20 %)
COPD	6	(15%)
Smoking	25	(62.5%)
Forced Vital Capacity (%) (median, SD)	101	(19)
Forced Expiratory Volume 1 sec (%) (median, IQR)	96	(84 to108)
FEV1/VC (median, SD)	75.9	(10.5)

Table 1. Continued

Characteristics		N=40 patients	
Ante-Surgery performance state (n, %)			
ASA	I	0	(0 %)
	II	5	(12.5 %)
	III	33	(82.5 %)
	IV	2	(5 %)
LVEF	good LVEF > 55%	25	(62.5 %)
	Reasonable LVEF 40-55 %	13	(32.5 %)
	Moderate LVEF 25-40%	1	(2.5 %)
	Poor LVEF < 25%	1	(2.5 %)
EuroSCORE (logistic (median, IQR)		4.7	(2.8 to 9.3)
Surgical parameters			
Surgical procedure			
	CABG	17	(42,5 %)
	CABG + single valve	2	(5 %)
	single valve	5	(12,5 %)
	multiple valve	4	(10 %)
	thoracic Aorta surgery (+/- valve +/- CABG)	12	(30 %)
Surgical duration			
	Surgery (min) (median, IQR)	312.5	(264.3 to 383.8
	Cardiopulmonary bypass (min) (median, IQR)	184.5	(132.3 to 227.3)
Intraoperative Steroid use		7	(17.5%)

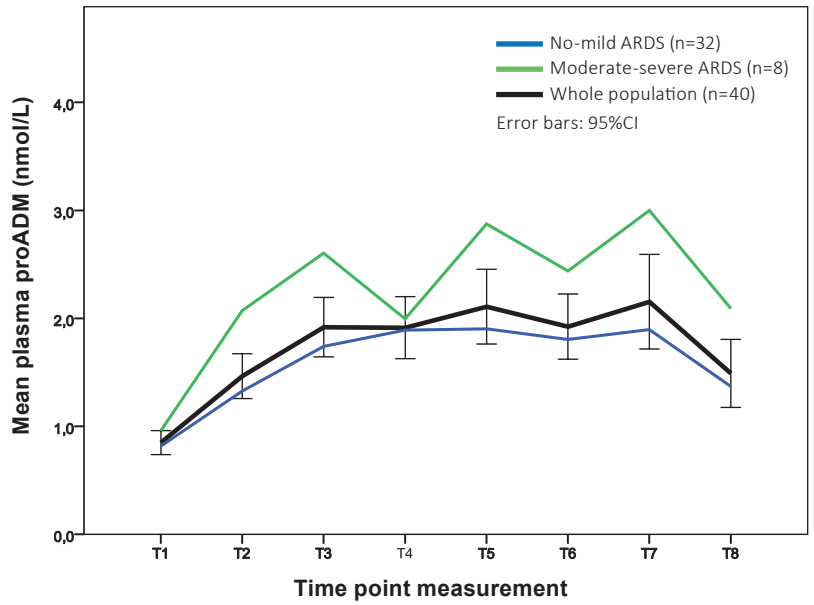
The incidence of pulmonary injury was 16/40 (40%); a mild lung reaction was present in 8/40 patients (5%), moderate ARDS in 6/40 (15%) and severe ARDS in 2/40 (5 %) (table 2). One out of eight patients with moderate to severe ARDS was pre-surgery known with a poor cardiac function. Two patients needed ultrafiltration to prevent fluid overload when weaning form the CPB. The chest X-ray abnormalities were in all patients but one present shortly after ICU-admittance. A decrease in p/F ratio developed from directly after ICU admission to 3 days after surgery. One out of eight patients with moderate to severe ARDS needed mechanical ventilation for more than 24 hours. Two patients died; one on day 45 after surgery due to an exacerbation of underlying pulmonary fibrosis, and one within the first week after surgery, due to cardiac failure.

Table 2. Incidence of major clinical outcomes

Outcome		
	n	***(%)***
ARDS (< 7 days after surgery)		
No	24	(60)
Mild	8	(20)
Moderate	6	(15)
Severe	2	(5)
Mortality	2	(5)
	Median	**IQR**
LOS-ICU (hours)	23	(21.3 to 45.8)
LOS-hospital (days)	9.8	(6.0 to 9.8)

The time course of mean proADM is given in figure 1. A significant increase of proADM after cardiac surgery was evident at all postoperative time-points as compared with preoperative baseline, but the steepest rise occurred between T1 (directly before start surgery) and T3 (at ICU arrival), i.e. the increase of proADM amounted to, on average 1.069 nmol/l (95% CI 0.835-1.305).

Figure 1. Mean absolute level of venous proADM in time



Mean post-surgery proADM levels were higher in the patients that developed ARDS as compared to those that did not (figure 2, supplement Table S5.1) and as well in patients with longer LOS-ICU. The perioperative change in proADM (i.e change between "directly before start of surgery" and "directly at admission at ICU") significantly predicted ARDS occurring within 7 days (OR 3.55 (95% CI 1.17 - 10.84), LOS-ICU > 48hr (OR 30.03 (95% CI 11.73 - 48.34) and LOS-hospital > 7days (OR 4.59 (95% CI 1.37 - 7.82) (figure 2). With respect to a cut-off value of T1-T3 proADM, a change of 1.50 nmol/L showed a specificity of 62.5 % and a sensitivity of 81.3 % for predicting ARDS within 7 days after cardiac surgery (supplement Table S5.2).

When the change in proADM (T1-T3) was added to the EuroSCORE in a predictive model its C-statistic improved over that of EuroSCORE alone. This was significant for ARDS and LOS-ICU>48hr (figure 3). Moreover, the predictive capacity of EuroSCORE plus proADM-change (T1-T3) was significantly better compared to that of EuroSCORE plus single-value proADM. Interestingly, the C-statistic of perioperative proADM-change (T1-T3) solely was better than the predictive value of EuroSCORE alone for all clinical outcomes.

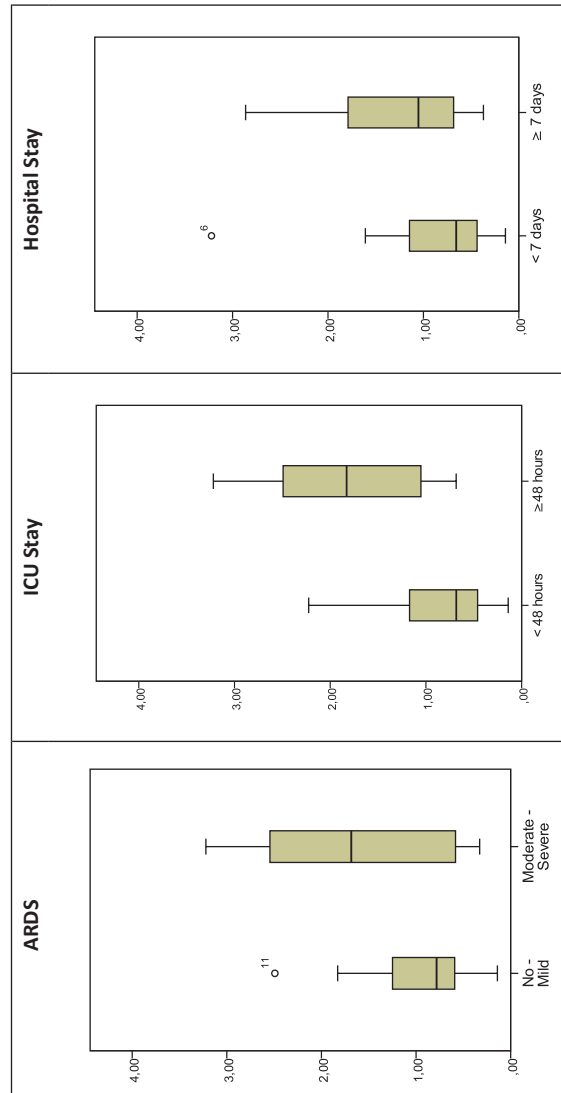
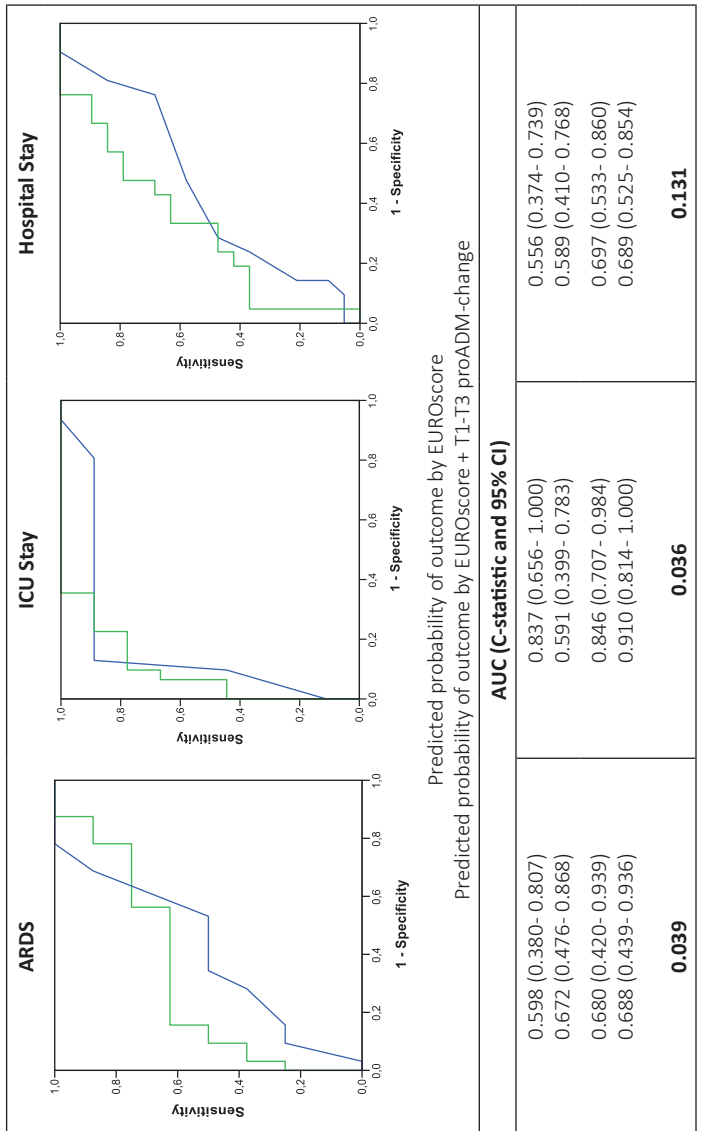
T1-T3 increase of venous plasma proADM (nmol/L)	Mean Difference 95% CI p-value	Odds Ratio 95% CI p-value
	0.389 -0.071- 0.850 0.400 0.974 0.501- 1.447 0.044 0.721 0.174- 1.268 0.004	4.59 1.37- 7.82 0.006 30.03 11.73- 48.34 0.002 3.55 1.17- 10.84 0.026

Figure 2. T1-T3 proADM-change in major clinical outcomes

Figure 3. ROC-curves and comparative AUC's of EuroSCORE +/- T1-T3 proADM-change for major clinical outcomes



	AUC (C-statistic and 95% CI)		
- EuroSCORE	0.598 (0.380- 0.807)	0.837 (0.656- 1.000)	0.556 (0.374- 0.739)
- T1-proADM	0.672 (0.476- 0.868)	0.591 (0.399- 0.783)	0.589 (0.410- 0.768)
- T1-T3 Δ proADM	0.680 (0.420- 0.939)	0.846 (0.707- 0.984)	0.697 (0.533- 0.860)
- EuroSCORE + T1-T3 Δ proADM	0.688 (0.439- 0.936)	0.910 (0.814- 1.000)	0.689 (0.525- 0.854)
*p-value**	**0.039**	**0.036**	**0.131**

**p value for difference of C-statistic of EuroSCORE +T1-T3 Δ proADM compared to EuroSCORE solely*

DISCUSSION

The main finding of this study is that in patients undergoing cardiac surgery the increase in proADM from just before surgical incision to time of arrival at ICU, significantly and independently predicted ARDS and increased LOS-ICU > 48 hr and LOS-hospital > 7days. Interestingly, although as single indicator a better predictor than EuroSCORE, the pre-to-post-operative change in proADM added robustness to the established clinical risk assessment score, the EuroSCORE, in predicting unfavorable clinical course after cardiac surgery. Based on a pre-to-post-surgical rise of proADM of > 1.5 nmol/l, we can faster than currently identify the possible patients at higher risk for development of ARDS and increased ICU-stay. This study moreover shows the impact of a cardiothoracic procedure on a patient (additional file 1); the mean proADM in our population at ICU arrival of 1.8 nmol/l equals that of patients admitted at the ICU with sepsis (25,26). We demonstrated a mean perioperative proADM-change of 1.069 nmol/l (95% CI 0.835 to 1.305). To our knowledge, our study is the first to show proADM-change as a clinically useful biomarker in postoperative care. Moreover, it contributes to knowledge on which time-points perioperative proADM values are most informative and sampling is worthwhile. Further studies, however, are necessary to confirm and assess an optimal perioperative proADM-change cut off point for predicting impaired clinical outcomes.

So far, the predictive value of single proADM measurements has been established in congestive and ischaemic heart diseases (27-30), in sepsis (25,26,31) in pulmonary diseases (32,33) and in patients developing ARDS (34). In cardiac surgery patients it was demonstrated that pre-operative values of proADM improve the predictive value of EuroSCORE (11) and post-operative proADM values, with a cut-off of 3.2 nmol/L, surpass predictive capacity of ICU APACHE IV score for mortality (35). Our study expanded on these prior observations by showing the value of consecutive perioperative proADM levels in predicting ARDS and LOS-ICU > 48hr and LOS-hospital > 7days after cardiac surgery. Up to a certain extent, baseline proADM concentrations can be viewed as marker of the pre-surgical physical state and morbidity (27,29,36). The pre-to- post-surgical rise in proADM, however, does justice to both the pre-surgical physical state and the magnitude of the impact of the whole procedure.

Additionally, from a pathophysiological angle, proADM seems a plausible indicator of the mechanisms behind development of ARDS after cardiac surgery. Namely, ADM is a long known potent vasodilator (12,37), and immunomodulator (13,14), and some studies report higher levels in the pulmonary artery compared to aorta (12,38,39,40). As ADM is expressed in many cell types in the lungs, including bronchial epithelium, bronchial smooth muscle, pulmonary vasculature and macrophages (16), ADM could,

by reflecting the local pulmonary inflammatory response, be implicated locally in the causal chain of events leading to priming for and development of inflammation and lung injury and even ARDS after cardiac surgery. But, since this study was not designed to confirm or refute this hypothesis, exploration of an etiologic association needs further investigation.

Our study has some limitations. First of all, our sample size is small (n=40) and the incidence of major end points low. However, the occurrence of ARDS in this study was similar to the incidence of ARDS as found in literature (4,5) Notwithstanding 8 of 40 patients developing moderate to severe ARDS, it is still not possible to build a predictive model with more than two parameters without the danger of over fitting. As alternative, we focused on the established EuroSCORE and showed an additive predictive value when the proADM-change was added to the EuroSCORE set of parameters. Secondly, the chosen proADM-change cut-off point of 1.5 nmol/L was based only on this small population. It would need more extensive testing in a different larger population before it can be reliably used in the clinical practice. Thirdly, the secondary outcomes were dichotomized into LOS-ICU > 48hr and LOS-hospital > 7days; we selected these cut off points on the basis of the opinion of an expert panel before analysis of the data. And furthermore, in our study population the EuroSCORE showed low prediction with only a c-statistic of 0.598 for ARDS and a c-statistic for hospital stay > 7 days of 0.556. In the original study by Nashef (9) the c-statistic was 0.759 for mortality and EuroSCORE was not validated for prediction of other outcome measures. Finally, it should be emphasized that we made a combination with the EuroSCORE and not with the in 2011 launched EuroSCORE II, which was not possible in our institution while mobility data and Canadian Coronary Score (CCS) were not available.

CONCLUSION

In conclusion, we demonstrated that the change in perioperative proADM levels were associated with ARDS and LOS-ICU > 48hr and LOS-hospital > 7days in critically ill patients after thoracic surgery and contributes significantly to the predictive value of the EuroSCORE. Employing the two time point measurements of proADM is promising in identifying patients at high risk for development of ARDS and complicated ICU and hospital stay early. Furthermore, it allows the clinician to focus and direct their lung-protective interventions to prevent or minimize secondary pulmonary injury after cardiac surgery in critically ill patients at the intensive care unit (ICU). However, it would need more testing in a different population before an actual clinically relevant cut off point can be set. And also, how proADM is causally related to the development of ARDS after cardiac surgery remains to be investigated and should be focus for future research.

LIST OF ABBREVIATIONS

ADM:	ADrenoMedullin
ARDS:	Adult Respiratory Distress Syndrome
ASA:	American Standards Association
CABG:	Coronary Artery Bypass Grafting
CCS:	Canadian Coronary Score
CPT:	Cardiopulmonary Bypass
EPD:	Electronic Patient Dossier
ICU:	Intensive Care Unit
LOS:	Length Of Stay
LV:	Left Ventricular
NYHA:	New York Heart Association
TRACE:	Time Resolved Amplified Cryptate Emission
TEE:	Trans Esophageal Echocardiography

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SUPPLEMENTARY MATERIAL

Contains:

Table S5.1 ProADM levels in time related to baseline and preceding values

Table S5.2 Sensitivity and specificity of T1-T3 ProADM-change predicting moderate to severe ARDS within 7 days after cardiac surgery.

PART THREE

Preventing lung injury after cardiac surgery

6

Physician’s preference-based instrumental variable analysis: is it valid and useful in a moderate-sized study?

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PMID: 25051311

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Jan P. Vandenbroucke, Saskia le Cessie, and Olaf M. Dekkers.

https://journals.lww.com/epidem/Fulltext/2014/11000/Physician_s_Preference_based_Instrumental_Variable.23.aspx

ABSTRACT

Background: Instrumental variable methods can potentially circumvent the unmeasured confounding inherent in observational data analyses.

Methods: We investigated the validity and usefulness of physician's preference instrumental variable analysis in the setting of a moderate-sized clinical study. Using routine care data from 476 elective cardiac surgery patients, we assessed the effect of preoperative corticosteroids on mechanical ventilation time and duration of intensive care and hospital stay, occurrence of infections, atrial fibrillation, heart failure, and delirium.

Results: Although results of the physician's preference-based instrumental variable analysis corresponded in direction to results of a recent large, randomized trial of the same therapy, the instrumental variable estimates showed much larger effects with very wide confidence intervals.

Conclusion: The lesser statistical precision limits the usefulness of instrumental variable analysis in a study that might be of sufficient size for conventional analyses, even if a strong and plausible instrument is available.

INTRODUCTION

Instrumental variable analysis can potentially circumvent confounding by indication that exists because of unknown or poorly recorded factors in observational data of anticipated therapy effects (1).

Physician's prescribing preference is a promising instrument because differences among physicians in therapy preferences are ubiquitous. We used anesthesiologist's preference in an instrumental variable analysis to investigate whether preoperative high dose corticosteroids are beneficial in cardiac surgery patients because they suppress the procedure-induced inflammatory response (2,3).

We compared instrumental variable analyses to standard regression techniques and to results from the recent Dexamethasone for Cardiac Surgery randomized trial (4).

METHODS

We used clinical data collected in the context of routine clinical care. The Leiden University Medical Centre review board waived the need of formal ethical approval and written informed consent.

Study Population

We assessed data on all adult patients who underwent elective cardiac surgery in the Leiden University Medical Centre in 2005. Patients had undergone a range of interventions, including coronary artery bypass grafting, valve repair/ replacement, and heart failure surgery. Patients treated with corticosteroids before admission for cardiac surgery were excluded, leaving 476 patients, of whom 115 received prophylactic corticosteroids. All received regular care according to the fast-track protocol (5).

Study End Points

Data on demographic features, type of surgical intervention and EuroSCORE were extracted from electronic and paper patient records. The EuroSCORE is a validated prognostic score of in-hospital mortality, based on patient-related, cardiac-related, and operation-related factors (6,7). Primary endpoints were 30-day mortality, ventilation time, and durations of intensive care unit (ICU) and hospital stays. Secondary outcomes were atrial fibrillation, infections, heart failure, delirium, norepinephrine use, glucose, and leukocyte count.

Statistical Analysis

We first used linear regression to estimate the effect of corticosteroids on the outcomes. This included crude analyses, multivariable analyses (adjusting for age, sex, diabetes, EuroSCORE, and type of surgery), and propensity score adjusted analyses (including the variables in the multivariable model plus the surgeon). Next, we performed 2-stage least squares instrumental variable analysis, with robust standard errors for dichotomous outcomes. The instrument was the proportion of all earlier patients of the same anesthesiologist who received corticosteroids. We selected this instrument based on the first-stage F-statistic and partial r^2 and on the range of predicted treatment probabilities. IV analyses were based on 461 patients (excluding 3 patients with unknown anesthesiologists, the only 2 patients of 1 anesthesiologist, and all first patients of the 10 anesthesiologists). Instrumental variable assumptions for our study were as follows (supplemental figure S6.1): (a) anesthesiologist's preference affects the probability that a patient receives corticosteroids; (b) anesthesiologist's preference for corticosteroids does not affect the outcome other than through the decision whether to administer corticosteroids, and (c) anesthesiologist's preference for corticosteroids is not related to characteristics of his patient population (8,9). The fourth assumption, required to obtain a point estimate (10,11), was the monotonicity assumption: no anesthesiologist would give corticosteroids to a certain patient unless all anesthesiologists with the same or a stronger preference would also give corticosteroids to that patient. The causal effect estimated is a local average treatment effect (11), a weighted average of the treatment effects in patients who would receive corticosteroids from anesthesiologists with a certain preference level, but not from anesthesiologists with a lower preference (10). Statistical analyses were performed with Stata 12 and the extension ivreg2 (12). Additional information regarding study population, data-extraction, study endpoints, conventional analyses, instrumental variable analyses, and sensitivity analyses are available at supplement Chapter 6.1.

RESULTS

Table 1 displays patient characteristics and outcomes according to received treatment. The EuroSCORE was higher in patients who received corticosteroids, suggesting confounding. For the selected instrument the first-stage F-statistic was 126 and the partial r^2 was 0.22 (see supplement Chapter 6.1 and Table S6.1). Table 2 shows patient characteristics across physician's preference quintiles. There was no clear pattern across physician's preference quintiles in EuroSCORE (see supplement Figure S6.2) for EuroSCORE per anesthesiologist) or other patient characteristics, suggesting physicians' preference for corticosteroids was not related to differences in patients' prognosis.

Table 1. Patient Characteristics and Outcomes by Treatment Status

	Prophylactic Corticosteroids			
	No. (n=361)		No. (n-116)	
Patient Characteristics				
Male	246	(68)	69	(60)
Age (years); mean (SD)	64.5	(13.5)	63.9	(12.9)
BMI (kg/m^2); mean (SD)	26.6	(4.2)	26.4	(4.2)
Diabetes mellitus	54	(15)	15	(13)
EuroSCORE; median (IQR)	4	(2–8)	5	(3–10)
EuroSCORE category				
1–2%	115	(32)	23	(20)
3–5%	110	(31)	35	(31)
≥ 6%	134	(37)	55	(49)
Type of Surgery				
Off-pump CABG	36	(10)	6	(5)
On-pump CABG	100	(28)	29	(25)
Valve	116	(32)	39	(34)
Combination/other	109	(30)	41	(36)
Outcomes				
Mortality (30 days)	10	(2.8)	4	(3.5)
Ventilation time (hrs); median (IQR)	10	(7–19)	11	(7–20)
ICU stay (days); median (IQR)	1	(1–3)	2	(1–4)
Hospital stay (days); median (IQR)	7	(6–11)	8	(6–13)
Highest norepinephrine dos > 0,1 µg/kg/min	112	(33)	35	(32)
Highest glucose (mmol/l); Mean (SD)	10.4	(2.4)	11.4	(2.5)
Highest leukocyte count (10^9/L); mean (SD)	13.4	(4.0)	15.6	(5.1)
Atrial fibrillation	173	(48)	50	(44)
Infection	52	(15)	15	(13)
Heart failure	48	(13)	22	(19)
Delirium	54	(15)	20	(18)

aNo. (%), unless otherwise indicated.

BMI, body mass index; CABG, coronary artery bypass graft; IQR, interquartile range

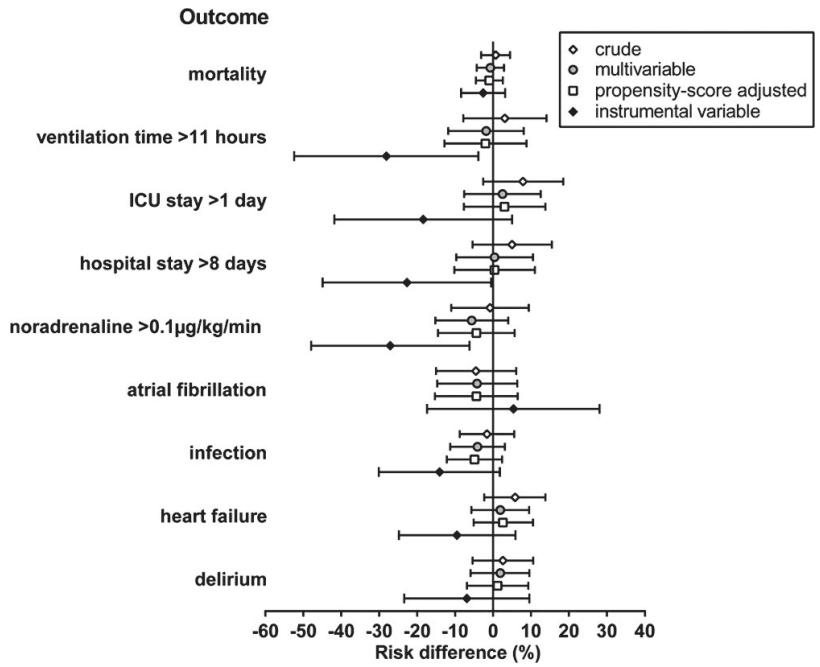
Table 2 shows a decreasing pattern across physician's preference categories for duration of ventilation and infections.

Table 2. Patient characteristics and Outcomes by instrumental Variable Status

	Instrumental Variable Qiuintile									
	1		2		3		4		5	
	(n=104)		(n=84)		(n=89)		(n=92)		(n=92)	
% Prophylactic corticosteroids in all previous patients	0		2-8		9-19		19-42		43-100	
Prophylactic corticosteroids in current patient	8	(8)	2	(4)	19	(21)	27	(29)	57	(62)
Patient Characteristics										
Male	69	(66)	52	(62)	59	(66)	63	(68)	61	(66)
Age (years); mean (SD)	62.6	(13.6)	65.2	(12.9)	65.1	(13.6)	64.0	(13.4)	63.8	(13.1)
BMI (kg/m^2); mean (SD)	25.9	(3.7)	26.5	(4.2)	26.4	(4.2)	26.3	(3.8)	27.1	(4.6)
Diabetes mellitus	14	(14)	9	(11)	13	(15)	11	(12)	16	(17)
EuroSCORE; median (IQR)	4	(2–9)	4	(2–8)	5	(3–8)	4	(2–9)	4	(2–9)
EuroSCORE category										
1–2%	34	(33)	28	(33)	18	(20)	29	(32)	28	(31)
3–5%	27	(26)	22	(26)	28	(32)	28	(30)	32	(35)
≥6%	41	(40)	34	(40)	42	(48)	35	(38)	31	(34)
Type of surgery										
Off-pump CABG	8	(8)	10	(12)	9	(10)	8	(9)	7	(8)
On-pump CABG	26	(25)	25	(30)	15	(17)	27	(29)	28	(30)
Valve	32	(31)	30	(36)	35	(39)	23	(25)	32	(35)
Combination/ Other	38	(37)	19	(23)	30	(34)	34	(37)	25	(27)
Outcomes										
Mortality (30 days)	2	(1.9)	2	(2.4)	5	(5.6)	4	(4.4)	1	(1.1)
Ventilation time (hrs); median (IQR)	12	(8–20)	11	(7–23)	12	(7–24)	10	(7–20)	9	(6–14)
ICU stay (days); median (IQR)	2	(1–4)	1	(1–3)	2	(1–3)	2	(1–4)	1	(1–1)
Hospital stay (days); median (IQR)	8	(6–13)	7	(5–9)	8	(6–11)	8.5	(6–14)	8	(5.5–9)
Highest norepinephrine dose > 0.1µg/kg/min	28	(31)	30	(37)	32	(39)	39	(45)	14	(16)
Highest glucose (mmol/l); mean (SD)	10.6	(2.3)	10.6	(2.5)	10.6	(3.0)	10.2	(1.8)	11.2	(2.6)
Highest leukocyte count (10^9/L); mean (SD)	13.0	(3.3)	12.8	(3.3)	14.7	(5.2)	14.4	(4.9)	15.0	(4.8)
Atrial fibrillation	42	(41)	40	(48)	45	(51)	44	(48)	44	(48)
Infection	18	(18)	16	(19)	12	(13)	12	(13)	9	(10)
Heart failure	18	(17)	9	(11)	19	(21)	14	(15)	9	(10)
Delirium	16	(16)	15	(18)	16	(18)	16	(18)	9	(10)

[a]No. (%), unless otherwise indicated.

Figure 1. Estimates of the effect of prophylactic corticosteroids on clinical outcomes in cardiac surgery patients, from crude, multivariable, propensity-score-adjusted, and instrumental variable analyses. Risk differences with 95% confidence interval are shown.



Results of conventional and instrumental variable analyses are displayed in the Figure 1 (dichotomous outcomes only) and supplement Table S6.2.

In general, unadjusted conventional analyses showed poorer outcomes in patients treated with corticosteroids (except for atrial fibrillation, infections, and norepinephrine dose). Multivariable and propensity-score-adjusted analyses generally showed a null effect. Instrumental variable results indicated a decreased risk of adverse outcomes (except atrial fibrillation) after corticosteroid administration. However, confidence intervals of IV estimates were much wider than those of conventional estimates. For example, crude analysis indicated the risk of a ventilation time >11 hours was 3.1% higher (95% confidence interval = −7.8% to 14.1%), propensity-score-adjusted analysis indicated it was 2.0% lower (−12.8% to 8.8%), and instrumental variable analysis indicated it was 28.1% lower −52.4% to −3.9%) for patients who received corticosteroids. Instrumental variable estimates of differences in glucose and leukocyte count were slightly higher than estimates from the other analyses (Supplement Table S6.2).

Because of our small sample size, we could compare our results only to secondary outcomes of the Dexamethasone for Cardiac Surgery randomized clinical trial (4). In general, effects in our instrumental variable analyses were similar in direction to the randomized clinical trial results (supplement Chapter 6.2) but with considerably larger effect sizes. For example, whereas our instrumental variable analyses estimated the risk of a ventilation time >24 hours to be 16.3% lower (−33.2% to 0.5%) for patients who received corticosteroids, the randomized clinical trial estimated this difference to be −1.5% (−2.7% to −0.3%) (4).

Neither adjusting the instrumental variable analysis for patient characteristics, nor using an instrumental variable based on the last 5 patients materially changed the results (supplement Table S6.3) Sensitivity analyses estimating relative risks yielded similar effect sizes (supplement Table S6.4).

DISCUSSION

We investigated whether physician's preference-based instrumental variable analysis was valid and useful in a moderate- sized study for the question whether preoperative corticosteroids are beneficial in cardiac surgery. In contrast to crude and propensity score-adjusted analyses, instrumental variable analysis using anesthesiologists' preferences as an instrument showed beneficial effects, similar in direction to the Dexamethasone for Cardiac Surgery randomized clinical trial results (4), and compatible with pathophysiologic insights concerning prevention of operation-induced systemic inflammation (13–15). However, compared with the trial results, the instrumental variable estimates were extremely large and confidence intervals were so wide as to preclude useful conclusions.

A reason for the difference in magnitude between our instrumental variable estimates and the randomized clinical trial results could be effect modification because of baseline prognostic differences between the study populations. Our patients seemed to be more high risk, as indicated by longer ventilation and ICU stay times and higher incidences of most outcomes.

There are also design-inherent explanations for the large size of the instrumental variable effect estimates. First, our smaller number of patients, compared with the randomized clinical trial, gives rise to less statistical precision, which is further aggravated in the IV analysis because of its 2-stage approach (16). This lack of precision, reflected in the large confidence intervals, could lead to the instrumental variable estimates being more extreme by chance.

Second, main instrumental variable assumptions may be violated. We would not expect differences in patient characteristics depending on anesthesiologist's preference for corticosteroids (independence assumption), as patients are assigned to the anesthesiologist on duty on the day of surgery. The lack of a consistent pattern in measured patient characteristics across quintiles of the instrumental variable is therefore reassuring. The assumption that preference for corticosteroids does not affect outcomes other than through administration of corticosteroids is more difficult to assess but seems plausible, as anesthesiologists took care of the patients only during surgery and were not involved in subsequent ICU care.

Third, violation of the monotonicity assumption could contribute to the extreme estimates. For example, if patients who receive corticosteroids from an anesthesiologist with a weak preference would not receive them from an anesthesiologist with a strong preference and if corticosteroids are of relatively little benefit to these patients, then the estimate of the effect of corticosteroids would be too favorable.

Fourth, estimands of the conventional and the instrumental variable analyses are different: the conventional analyses estimate average treatment effects in the population, whereas the instrumental variable analyses estimate local average treatment effects (as explained in the Methods section).

Fifth, finite sample bias might be a reason for the large instrumental variable effect estimates. However, the first-stage F-statistic of 126 should be sufficient for finite sample bias to be negligible (1). We further explored this use of simulations under conditions similar to our study (100–500 patients; mean partial r^2 of 0.17; unmeasured confounding and a binary outcome occurring in 50% of patients; see supplement. Mean instrumental variable estimates were close to the "true" treatment effect of 0.10, even when the sample size was reduced to 100 patients, indicating no substantial finite sample bias with an instrument of this strength.

CONCLUSION

In conclusion, despite availability of a strong instrument, plausibly fulfilling main instrumental variable assumptions, physician's preference-based instrumental variable analysis in a moderate-sized study population showed results that differed greatly in magnitude from results of a major randomized clinical trial on the same intervention. We have explored possible reasons and conclude that this phenomenon is most likely because of the reduced statistical precision of the instrumental variable analysis in datasets of moderate size.

Acknowledgements

We thank Stefan Dieleman of the DECS Study Group for providing additional information on their data, which enabled us to compare our data to data from a randomized clinical trial on the same question. We also thank Michel Versteegh for supplying the EuroSCORE data for the study population.

LIST OF ABBREVIATIONS

ICU: Intensive Care Unit
IV: Instrumental Variable

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SUPPLEMENTARY MATERIAL

Contains:

7

The efficacy and safety of prophylactic corticosteroids for the prevention of adverse outcomes in patients undergoing heart surgery using cardiopulmonary bypass: a systematic review and meta-analysis of randomized controlled trials

Eur J Cardiothorac Surg. 2020 Apr 1;57(4):620-627. doi: 10.1093/ejcts/ezz325.
PMID: 31972000.

Ng KT, Van Paassen J, Langan C, Sarode DP, Arbous MS, Alston RP, Dekkers OM.

ABSTRACT

Corticosteroids are often administered prophylactically to attenuate the inflammatory response associated with cardiac surgery using cardiopulmonary bypass (CPB). However, the efficacy and safety profile of corticosteroids remain uncertain.

The primary aim of this systematic review and meta-analysis was to investigate the effect of corticosteroids on mortality in adult cardiac surgery using CPB. Secondary aims were to examine the effect of corticosteroids on myocardial adverse events, pulmonary adverse events, atrial fibrillation, surgical site infection, gastrointestinal bleeding, and duration of stay in the intensive care unit and hospital. Randomized controlled trials (RCTs) were systematically searched in electronic databases (MEDLINE, EMBASE, CINAHL, CENTRAL and Web of Science) from their inception until March 2019. Observational studies, case reports, case series and literature reviews were excluded.

Sixty-two studies (n = 16 457 patients) were included in this meta-analysis. There was no significant difference in mortality between the corticosteroid and placebo groups (odds ratio (OR) 0.96, 95% confidence interval (CI) 0.81-1.14; P = 0.65, participants = 14 693, studies = 24, evidence of certainty: moderate). Compared to those receiving a placebo, patients who were given corticosteroids had a significantly higher incidence of myocardial adverse events (OR 1.17, 95% CI 1.03-1.33; P = 0.01, participants = 14 512, studies = 23) but a lower incidence of pulmonary adverse events (OR 0.86, 95% CI 0.75-0.98; P = 0.02, participants = 13 426, studies = 17). The incidences of atrial fibrillation (OR 0.87, 95% CI 0.81-0.94; P < 0.001, participants = 14 148, studies = 24) and surgical site infection (OR 0.81, 95% CI 0.73-0.90; P < 0.001, participants = 13 946; studies = 22) were all lower in patients who were given corticosteroids.

In the present meta-analysis of 62 RCTs (16 457 patients), including the 2 major RCTs (SIRS and DECS trials: 12 001 patients), we found that prophylactic corticosteroids in cardiac surgery did not reduce mortality. The clinical significance of an increase in myocardial adverse events remains unclear as the definition of a relevant myocardial end point following cardiac surgery varied greatly between RCTs.

INTRODUCTION

Based on data from the Society of Thoracic Surgeons Adult Cardiac Surgery Database, 292 500 patients underwent myocardial revascularization and/or heart valve replacement in 2017 (1). The introduction of cardiopulmonary bypass (CPB) in the early 1950s revolutionized heart surgery (2,3). However, CPB often induces a systemic inflammatory response syndrome (SIRS) where at least 2 or more systemic inflammatory response syndrome criteria were met by nearly 95% of patients within the first day after cardiac surgery (4–8). Systemic inflammatory response syndrome involves complement activation, along with activation of platelets, neutrophils, monocytes and macrophages (5,9). As a result, coagulation and fibrinolytic cascades are initiated (6,9). The ensuing systemic inflammatory response is associated with fever, impaired alveolar gas exchange, vasodilatation, myocardial stunning, renal insufficiency and multi-organ dysfunction (4,10–12). Adverse outcomes from heart surgery including myocardial infarction, pulmonary dysfunction, renal impairment and death are associated with systemic inflammatory response syndrome (13–15).

Corticosteroids are potent anti-inflammatory agents, which inactivate inflammatory genes and inhibit synthesis of anti-inflammatory proteins during the process of inflammation (5,16). They inhibit the release of biochemical inflammatory markers, minimizing the CPB-induced inflammatory response (5,16). In addition, generic corticosteroids are low-cost drugs, and as such more likely to be cost-effective if their use is associated with reduced incidences of adverse events after heart surgery with CPB. However, corticosteroids may have their own adverse effects. They commonly cause hyperglycemia, which has been associated with immunosuppression and poor wound healing (5,17,18). In addition, high-dose corticosteroids use have been associated with an increased risk of gastrointestinal bleeding (5,17). Whilst the anti-inflammatory effects of corticosteroids seem desirable, robust analysis on the evidence of efficacy and safety of corticosteroids is required before recommendations on the use of corticosteroids in heart surgery with CPB can be made.

A previous systematic review and meta-analysis published in 2011 found that corticosteroids were not associated with any significant reduction in clinically important adverse outcomes from heart surgery (19). Since that review was published, 8 randomized controlled trials (RCTs) including 2 trials (20,21) with large population sample sizes have been published (20–27). Thus, an updated systematic review and meta-analysis is warranted to summarize the current understanding of the use of corticosteroids in patients undergoing heart surgery with CPB.

The primary aim of this review was to determine the effect of prophylactic corticosteroids on mortality in adult cardiac surgery with CPB. Secondary aims were to examine the effect of corticosteroids on complications of adult cardiac surgery, such as myocardial adverse events (including fatal and non-fatal myocardial infarction), pulmonary adverse events (including pulmonary oedema, infection or prolonged postoperative ventilation for respiratory failure), atrial fibrillation, surgical site infection, gastrointestinal bleeding and duration of stay in the intensive care unit (ICU) and hospital.

METHODS

This review paper was conducted in accordance with the Cochrane Handbook for Systematic Reviews of Interventions (28). The research questions were formulated using a population intervention-comparison-outcomes approach (supplement Table S7.1).

Literature search

The Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (OvidSP), Embase (OvidSP), CINAHL (EBSCO), Science Citation Index Expanded (SCI-EXPANDED), Social Science Citation Index (SSCI) and Web of Science (Thomson Reuters) were searched (supplement Table S7.2), by Jacob Heeren user on 21 November 2022 until 31 March 2019, for RCTs comparing corticosteroids with either placebo or no treatment in adults undergoing heart surgery with CPB. The ClinicalTrials.gov (www.CLINICALTRIals.gov) and the WHO International Clinical Trials Registry Platform (ICTRP) Search Portal (http://apps.who.int/trial search/) databases were searched for any ongoing or unpublished trials. No restrictions on language of publication were applied. Reference lists from retrieved RCTs and systematic reviews and meta-analyses were hand-searched to identify any additional trials. Study authors were contacted for any missing or incomplete data when required.

Studies reporting parallel-arm RCTs were included in this review. There were no restrictions with regard to the duration of the study follow-up period. Studies comprising only off-pump coronary artery bypass grafting (CABG) surgery were excluded. However, studies that included both heart surgery with/and without CPB were included if data for those patients who underwent heart surgery with CPB, were reported separately. Studies involving pediatric populations were also excluded in this review because the harmful biological effects of CPB are more prominent in infants and newborns than the adult population, which may introduce a type II statistical error.

Outcomes

The primary outcome for this meta-analysis was mortality, where the data of the longest duration of follow-up were used for analysis. Secondary outcomes included postoperative myocardial adverse events (myocardial infarction based on either electrocardiography diagnosis, troponin-I, creatinine kinase-muscle/brain or lactate dehydrogenase), pulmonary adverse events (including pulmonary oedema, pleural effusion, pneumonia, pulmonary embolism and respiratory failure), surgical site infection (wound infection or mediastinitis), atrial fibrillation, gastrointestinal bleeding (ulcer, bleeding or perforation), resternotomy, stroke, author-defined acute kidney injury (increased creatinine level, oliguria or requiring dialysis), author-defined use of positive inotropes/vasopressor intraoperatively, requirement for blood transfusion and postoperative blood glucose level. Data from all available time points were recorded and for data analysis, the longest reported time-point was used for each study. Process outcomes that were evaluated included the duration of ICU stay (hours), duration of hospital stay (days) and quality of life (The Assessment of Quality of Life Scales, 5-Level EuroQol Health Survey, 36-Item Short-Form Health Survey).

Study selection

Selection of studies was conducted in adherence to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (29). Two review authors (C.L. and D.P.S.) independently screened titles and abstracts for eligibility. Studies were coded as 'retrieve' (eligible or potentially eligible) or 'do not retrieve' (not eligible). Any disagreements at this stage were resolved by a third author (K.T.N.). The full-text study reports of potentially eligible studies were retrieved. Two review authors (C.L. and D.P.S.) independently screened the full-texts, identified those studies for inclusion and recorded reasons for the exclusion of ineligible studies. Any disagreements were resolved through consultation with a third author (K.T.N.). Duplicates were excluded and multiple reports of the same study were collated so that each study rather than each report was the unit of interest in this review.

Data extraction

Data were extracted from the full-text article of each included study by 2 authors independently (C.L. and D.P.S.) using a standardized data-extraction form. Disagreements between the data extractors were resolved by involving a third author (K.T.N.). One review author (K.T.N.) transcribed the data into the Review Manager file and ensured that data had been entered correctly by comparing the extracted data with that in the study reports. A second review author (D.P.S.) spot-checked study characteristics for accuracy.

Risk of bias assessment

Two review authors (C.L. and D.P.S.) independently assessed the risk of bias for each study using the Cochrane Collaboration's Risk of Bias tool (28). Disagreements were resolved through discussion with a third author (K.T.N.). Each potential source of bias was graded as low, high or unclear. The risk of bias for each domain was summarized across all the included studies

Statistical analysis

Review Manager version 5.3 was used for statistical analyses (30). A 2-sided q-value of 60% were used to define low, moderate and substantial levels of heterogeneity, respectively. If no substantial heterogeneity was noted, a fixed effects model analysis was used to pool estimates. If substantial heterogeneity (I^2 >_ 60%) was observed, a random effects model analysis was used.

Subgroup analyses and sensitivity analysis

A funnel plot was created to explore the possibility of publication bias for the primary outcome. Subgroup analyses were performed on the major outcomes (mortality, myocardial adverse events, pulmonary adverse events, atrial fibrillation and surgical site infection), by stratifying dose of steroids into high-dose (total administered dose >1 g of hydrocortisone-equivalent) and low-dose (total administered dose total administered dose ≤1g of hydrocortisone-equivalent) (equivalent anti-inflammatory doses of corticosteroids: prednisolone 5mg=betamethasone 750µg; deflazacort 6mg; dexamethasone 750µg; hydrocortisone 20mg; methylprednisolone 4mg; prednisone 5mg; triamcinolone 4mg) (32). To assess the robustness of our primary outcome (mortality), we also performed a sensitivity analysis by including only studies of low risk of bias.

Certainty of evidence assessment

The GRADE assessments of the evidence and summary of findings were independently performed by 2 authors (D.P.S. and C.L.) using the GRADEpro/GDT software (31). Based on the Cochrane handbook, we downgraded a starting rating of 'high quality' evidence of RCT based on the 5 criteria (risk of bias, inconsistency, indirectness, imprecision and publication bias) by 1 level for serious concern or by 2 levels for very serious concerns. Any disagreements were resolved by a third author (K.T.N.).

Trial sequential analysis

Trial sequential analysis was performed on the primary outcome (mortality) to assess the risk of random error and multiplicity phenomenon due to repeated significant testing in meta-analyses (33). The required meta-analysis information size and adjusted significance thresholds were calculated based on a 2-sided sequential analysis-adjusted fixed effects model with 5% risk of type 1 error and power of 80%.

RESULTS

Trial selection

Searching of the databases found 9523 non-duplicate citations for titles and/or abstracts screening. Eighty relevant articles were retrieved for full-text assessment. Of these, a total of 62 studies (16 457 patients) were included in this present systematic review (Supplement Figure S7.1). The clinical characteristics of all included studies are illustrated in supplement Table S7.3. Searching of clinical trials registers identified 2 relevant ongoing studies (supplement Table S7.4) (34,35).

Study characteristics

Of the included 62 studies, 8 were published since 2010, 36 were in the 2000s, 11 in the 1990s, 4 in the 1980s and 3 in the 1970s. Altogether, 16.457 patients were included from 62 trials with a mean age of 65 years and a predominance of male participants (66.3%). Only 7 studies included specifically 'high-risk' surgical patients with all other study-populations consisting of 'low-risk' or 'unspecified-risk' CABG, heart valve or other heart surgery. In the majority of the studies, the sample sizes were small (median number of patients per study = 50). The type of corticosteroids (hydrocortisone (n = 7), methylprednisolone (n = 53), dexamethasone (n = 15), prednisone (n = 2), betamethasone (n = 1), combination of hydrocortisone and methylprednisolone (n = 1), combination of methylprednisolone and prednisone (n = 3)),period of treatment and dosage of corticosteroids administered varied widely between studies.

Risk of bias assessment

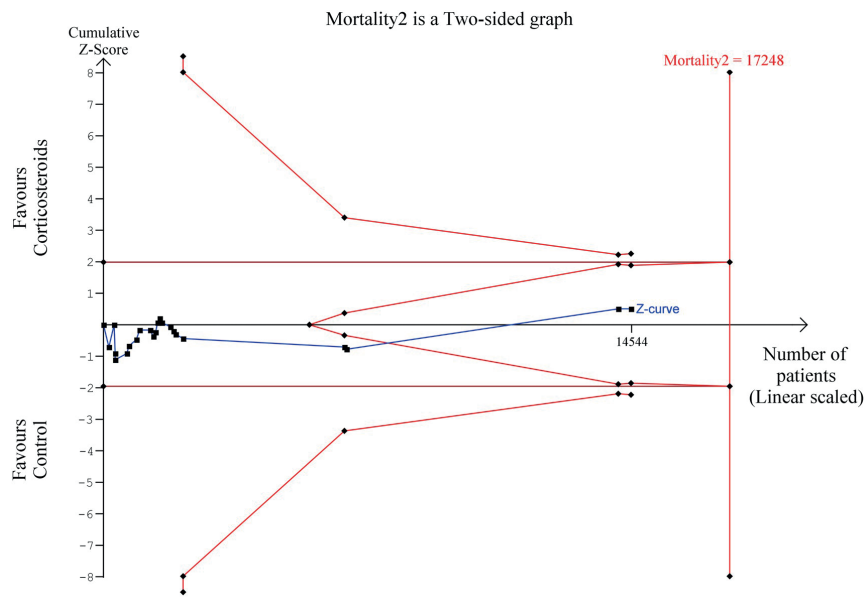
The summary risk of bias assessment was 'Low' for 19 studies, 'Unclear' for 27 studies and 'High' for 16 studies (Supplement Figure S7.2 and S7.3) The greatest source of bias across the studies was lack of blinding. The summary of findings/quality of evidence is displayed in supplement Table S7.5. Results of the meta-analyses for all primary and secondary outcomes are outlined in supplement Table S7.6.

Primary outcome

The use of corticosteroids in heart surgery did not reduce mortality (Peto OR 0.96, 95% confidence interval (CI) 0.81–1.14; participants = 14 693; studies = 24; certainty of the evidence: moderate). Statistical heterogeneity was low (I^2 = 0%). The funnel plots for mortality did not reveal substantial asymmetry, suggesting a low risk of publication bias. Sensitivity analysis of low-risk bias trials demonstrated similar results (Peto OR 0.95, 95% CI 0.79–1.13). The trial sequential analysis of a diversity-adjusted required information size for mortality was 17.248 patients (Figure. 1). With 14.693 patients, only 85.2% of the required information size was available to detect or reject a relative risk reduction of 20%, based on a 5% risk of type 1 error (2-sided), a power of 80%, and

an incidence in the control arm of 3.95% with a model variance based heterogeneity correction.

Figure 1. Trial sequency analysis of mortality**.**



Secondary outcomes

The risk of myocardial adverse events was significantly increased in patients receiving corticosteroids (Peto OR 1.17, 95% CI 1.03– 1.33; participants = 14 512, studies = 23, the certainty of the evidence: very low). The definition of myocardial adverse events used in the Steroids in Cardiac Surgery trial (20) differed from the other studies as it was based only on a rise in the cardiac enzyme (creatinine kinase-muscle/brain), which led to a very high incidence of myocardial adverse events (13% in corticosteroids group, 11% in the placebo group) (20). The combination of post hoc analysis on the incidence of myocardial infarction (defined as the presence of new Q-waves on the postoperative electrocardiograph) in the SIRS trial (20) and sub-analysis of the remaining studies showed no significant difference in myocardial adverse events between the corticosteroid and placebo groups (Peto OR 0.90, 95% CI 0.70–1.16; participants = 14 512, studies = 23; certainty of the evidence: very low), indicating the introduction of bias as a result of including the SIRS trial in this measured outcome. By removing the SIRS trial in the sensitivity analysis, the difference in the incidence of the myocardial adverse events became non-significant and the effect changed direction (Peto OR 0.91, 95% CI 0.68–1.20; participants = 7.005, studies = 22).

Corticosteroids significantly reduced the incidence of pulmonary adverse outcomes (Peto OR 0.86, 95% CI 0.75–0.98; participants = 13.426; studies = 17 studies, I^2 = 0%, the certainty of the evidence: low). In comparison to the placebo, the incidence of atrial fibrillation (Peto OR 0.87, 95% CI 0.81–0.94; participants = 14.148, studies = 24, certainty of the evidence: very low) and surgical site infections (Peto OR 0.81, 95% CI 0.73–0.90; participants = 13.946, studies = 22, certainty of the evidence: low) were significantly lower in the corticosteroids group. The duration of ICU stay was shorter in patients who received corticosteroids compared to a placebo (mean difference -4.41 h, 95% CI –6.13 to –2.70; participants = 13.490, studies = 31) as was the duration of hospital stay (mean difference –0.54 days, 95% CI -1.05 to -0.02; participants = 13.196, studies = 21). There were no significant differences between patients who received corticosteroids compared to placebo in the incidence of gastrointestinal bleeding (Peto OR 1.29, 95% CI 0.70–2.39; participants = 5.026, studies = 5), author-defined acute kidney injury (Peto OR 0.84, 95% CI 0.68–1.02; participants = 12.734, studies = 12), resternotomy (Peto OR 1.12, 95% CI 0.47–2.65; participants = 818, studies = 7), stroke (Peto OR 0.84, 95% CI 0.66–1.06; participants = 13.218; studies = 14), use of positive inotropes (Peto OR 0.98, 95% CI 0.74–1.30; participants = 1.390, studies = 19) or required for packed red cell transfusion (Peto OR 0.96, 95% CI 0.88–1.05; participants = 8.127, studies = 7).

There was no standardization on the reporting of postoperative blood glucose levels across all the included studies. Three studies recorded the highest postoperative blood glucose level (9,21,36), another 4 trials (20,23,37,38) reported the number of patients with postoperative hyperglycemia and 10 (18,25–27,39–44) reported different time-points of blood glucose level between the corticosteroid and placebo groups. Thus, a meta-analysis of postoperative blood glucose levels was not performed due to significant variation in the interpretation of glucose level across studies.

Subgroup analyses

Eleven studies (22,27,37,38,45–51) used low-dose corticosteroids with the remaining 51 studies administered high-dose corticosteroids. There was no significant interaction between high- and low-dose corticosteroid and the incidences of myocardial or pulmonary adverse events and surgical site infection. However, there was significant interaction (P = 0.001) with the incidence of atrial fibrillation, with the treatment effect favoring low- over high-dose corticosteroids. Covariate distribution occurred in this subgroup analysis due to an inadequate number of trials (6) and sample size (n = 924) with moderate heterogeneity in the low-dose corticosteroid subgroup

Quality of life

Since the earlier review, only 2 studies (21,32) investigated quality of life outcomes. In the Dexamethasone for Cardiac Surgery trial, using the SF-36 (physical and mental components), there was no clear difference between the corticosteroid and placebo groups (21). The outcomes of EQ-5D also remained similar between the 2 groups (21). One sub-study of SIRS trial utilized the Postoperative Quality of Recovery Scale to assess the quality of recovery after heart surgery (32). In 482 patients available for the recovery analysis, there were no differences between the corticosteroid and placebo groups for overall recovery and individual recovery domains (32).

DISCUSSION

In this updated systematic review and meta-analysis of the use of prophylactic corticosteroids in heart surgery with CPB, no effect on mortality could be demonstrated. This finding supports the recommendation of the 2017 EACTS Guidelines on Perioperative Medication in Adult Cardiac Surgery where the routine use of prophylactic corticosteroids is not indicated for adults undergoing cardiac surgery (Class of recommendation III and Level of Evidence A) (52). The present study includes 2 recent RCTs (DECS and SIRS trials), which both have very much larger sample sizes compared to earlier published RCTs (20,21). Both trials included mainly 'high risk' patients for heart surgery. As these 2 trials dominate the results of the meta-analysis, the evidence from the present systematic review and meta-analysis can be considered generalizable to the current population undergoing heart surgery, which commonly consists of elderly patients with multiple comorbidities In the subgroup analysis of the DECS trial based on the treatment-by-age interaction of corticosteroids on mortality events, it suggested that a younger patient age (<65 years) when receiving corticosteroids (21). It is possible that younger patients have a more intense inflammatory response than elderly patients where suppression of this effect with corticosteroids may contribute to a benefit in young patients (52). However, such treatment-by-age interaction was not observed in the SIRS trial (20). The sex-based subgroup differences of corticosteroids on mortality were not significant in both the DECS and SIRS trials (20,21). Given that the mortality risk after cardiac surgery was small (4%), we would need a trial with a large population sample size to prove an effect on mortality from corticosteroids. The present meta-analysis did not achieve the required population sample size to detect a 20% reduction in mortality based on a 5% risk of type 1 error (2-sided) and 80% power. Thus, the findings of this meta-analysis cannot reliably exclude that corticosteroids may influence mortality in patients undergoing heart surgery with CPB.

The higher incidence of myocardial adverse events in patients receiving corticosteroid needs to be interpreted with caution as different definitions of myocardial infarction were used across different RCTs. The majority of cases of myocardial complications came from the SIRS trial (20). In this study, the myocardial injury was defined as a rise in creatinine kinase muscle/brain levels above a predefined threshold and/or presence of new Q-waves on the postoperative electrocardiography due to limited access to troponin measurement in some centers (20). This may have contributed to the high levels of heterogeneity found in the analysis of myocardial adverse outcomes. Sensitivity analysis following the removal of data from the SIRS trial resulted in a major change in the direction of the effect and magnitude of the statistical finding. By including the reported incidence of myocardial infarction based on the post hoc analysis of the SIRS (20), the finding corresponded to the aforementioned sensitivity analysis that corticosteroids did not increase the risk of myocardial infarction. Despite a statistically significant increase in the incidence of myocardial adverse events in patients receiving corticosteroids in the present meta-analysis, the clinical significance of this finding is unclear as it was not associated with an increase in mortality. Heart surgery is associated with myocyte trauma from cardioplegia and surgical trespass of the myocardium, so biological markers will be released but will not always be associated with clinically relevant adverse myocardial outcomes. Therefore, defining what clinically relevant myocardial adverse events following heart surgery are, is challenging.

In the present systematic review and meta-analysis, the reduction of pulmonary complications, atrial fibrillation and surgical site infections along with shorter durations of ICU and hospital stay in patients receiving corticosteroids may indicate the limited value of mortality as an outcome where the disease-specific benefit is likely to be in other clinical outcomes (53). There remains scope for further investigation of patient recovery outcomes and inflammation-specific outcomes in future trials. Thus, an ongoing RCT (DECS-II study, NCT03002259) has been designed to examine the patient-centered outcomes, which focusses on enhanced recovery and earlier hospital discharge in adult patients after high-dose corticosteroids in heart surgery with CPB (single-dose administration of 1 mg/kg, maximal dose of 100 mg of dexamethasone before CPB) (35).

Given that the majority of sample size for incidences of atrial fibrillation and surgical site infection were contributed by two high quality large trials (DECS and SIRS), it is likely reflective of the true effect of prophylactic corticosteroids on the aforementioned outcomes (20,21). The positive effect of corticosteroids in reducing the incidence of atrial fibrillation and surgical site infection found in the present meta-analysis could be skewed by many of the previous RCTs with a small population sample size with a high risk of study bias and substantial heterogeneity. Thus, we confirmed that corticosteroids did not reduce the incidence of atrial fibrillation and surgical site infection based on the negative findings of the 2 robust large RCTs (20,21). However, patients with

chronic steroid therapy should continue their usual dose of corticosteroids on the day of operation (52,54). Additional preoperative stress–dose corticosteroids may be appropriate but is not evidence-based (54). The potential benefits of corticosteroids on these secondary outcomes warrant future adequately powered RCTs to establish the true effect on these postoperative outcomes.

The present systematic review and meta-analysis are incomparable with those undertaken prior to publication of the DECS and SIRS trials (14,15), because the sample sizes of these 2 trials are so much larger than all the previously published trials. The benefits of corticosteroids reducing mortality found in meta-analysis undertaken prior to these 2 large RCTs may have had false-positive signals due to small sample sizes and so the potential for a type I error. Another systematic review and meta-analysis have been published recently which included both recent large RCTs (20,21). It included 56 studies between 1977 and 2015 and concluded that corticosteroids had an unclear impact on mortality with an increased risk of myocardial injury (55). In the present systematic review, we have updated our literature search up to 2019 and included 11 studies (23–25,43,48,56–61) that were not included by Dvirnik et al.'s review (55). Moreover, 5 of the 56 studies were excluded from our review due to lack of randomization (62,63), the inclusion of children in the study population (64), the inclusion of patients undergoing non-heart surgery (65) or non-compatible study design (66). There were also some slight differences in our search strategy which may have led to different search outcomes between the 2 meta-analyses. In contrast to the latest meta-analysis (55), our findings suggest that the risk of myocardial adverse events may be overestimated and indeed, there may be some benefits on secondary outcomes, namely postoperative pulmonary complication and length of ICU/hospital stay from the use of corticosteroids that require future RCTs to confirm the certainty of evidence.

Limitations

There are several important qualitative limitations of the RCTs that were used in the present meta-analysis, which will have influenced the interpretation of our findings. Firstly, the risk of bias of most of the included RCTs was classified as either 'Unclear' or 'High' (43/62). Secondly, in many of the included RCTs, the primary end points were either surrogate markers of inflammation or ventilator parameters, and reporting of clinical outcomes did not form part of the study protocol. Non-standardized collection of clinical outcomes carries a high risk of observer bias, particularly when outcome adjudication is not blinded. Furthermore, the duration of follow-up periods were short and heterogeneous in the majority of RCTs, so inflating the risk of under-reporting of adverse outcomes. Thirdly, DECS and SIRS trials that contributed 12.001/16.457 patients and resulted in >80% of the pooled effects on the primary outcome (20,21).

Therefore, these 2 RCTs (20,21) will have heavily influenced both the mortality outcome and the risk of bias for this meta-analysis. As these 2 RCTs (20,21) were of high quality and had well-defined clinical outcomes as primary end points, as well as long periods of follow-up, some qualitative limitations discussed earlier will clearly be of less importance in this updated meta-analysis. Furthermore, 62 RCTs spanned across 4 decades, from the mid-1970s until 2017. During this time, many aspects of anesthesia, surgical and perioperative care have greatly changed. The type of cardiac surgery, study protocols and definitions of myocardial or pulmonary adverse events that were used, varied across all the included studies, which may have introduced variance into our findings. In this review, the very low to moderate level of evidence as a result of the risk of bias, inconsistency, imprecision and publication bias, limits any recommendations on the prophylactic use of corticosteroids in cardiac surgery with CPB.

CONCLUSION

In conclusion, in the present meta-analysis of 62 RCTs (16.457 patients), including of the 2 major RCTs (SIRS and DECS; 12 001 patients), prophylactic corticosteroids in cardiac surgery did not reduce mortality. The clinical significance of an increase in myocardial adverse events remains unclear as defining a relevant myocardial end point following cardiac surgery is challenging.

LIST OF ABBREVIATIONS

CABG:	Coronary Artery Bypass grafting
CI:	Confidence Interval
CPB:	Cardio Pulmonary Bypass
DECS:	The Dexamethasone for Cardiac Surgery trial
ICU:	Intensive Care Unit
OR:	Odds Ratio
RCTs:	Randomized Controlled Trials
SIRS trial:	Steroids In Cardiac Surgery trial

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SUPPLEMENTARY MATERIAL

Contains:

8

Corticosteroid use in COVID-19 patients: a systematic review and meta-analysis on clinical outcomes

Crit Care. 2020 Dec 14;24(1):696. doi: 10.1186/s13054-020-03400-9.
PMID: 33317589; PMCID: PMC7735177.

J. van Paassen, J.S. Vos, E.M. Hoekstra, K.M.I. Neumann, P.C. Boot, S.M. Arbous.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7735177/pdf/13054_2020_Article_3400.pdf

ABSTRACT

Background: In the current SARS-CoV-2 pandemic, there has been worldwide debate on the use of corticosteroids in COVID-19. In the recent RECOVERY trial, evaluating the effect of dexamethasone, a reduced 28-day mortality in patients requiring oxygen therapy or mechanical ventilation was shown. Their results have led to considering amendments in guidelines or already recommending corticosteroids in COVID-19. However, the effectiveness and safety of corticosteroids remain uncertain, and reliable data to further shed light on the benefit and harm are needed.

Objectives: The aim of this systematic review and meta-analysis was to evaluate the effectiveness and safety of corticosteroids in COVID-19.

Methods: A systematic literature search of RCTS and observational studies on adult patients was performed across Medline/PubMed, Embase and Web of Science from December 1, 2019, until October 1, 2020, according to the PRISMA guidelines. Primary outcomes were short-term mortality and viral clearance (based on RT-PCR in respiratory specimens). Secondary outcomes were: need for mechanical ventilation, need for other oxygen therapy, length of hospital stay and secondary infections.

Results: Forty-four studies were included, covering 20.197 patients. In twenty-two studies, the effect of corticosteroid use on mortality was quantified. The overall pooled estimate (observational studies and RCTs) showed a significant reduced mortality in the corticosteroid group (OR 0.72 (95%CI 0.57–0.87). Furthermore, viral clearance time ranged from 10 to 29 days in the corticosteroid group and from 8 to 24 days in the standard of care group. Fourteen studies reported a positive effect of corticosteroids on need for and duration of mechanical ventilation. A trend toward more infections and antibiotic use was present.

Conclusions: Our findings from both observational studies and RCTs confirm a beneficial effect of corticosteroids on short-term mortality and a reduction in need for mechanical ventilation. And although data in the studies were too sparse to draw any from conclusions, there might be a signal of delayed viral clearance and an increase in secondary infections.

INTRODUCTION

Since the start of the outbreak, Coronavirus disease 2019 (COVID-19), caused by the novel coronavirus SARS-CoV-2, has spread globally from Wuhan, China. 40,559,736 cases have been reported and 1,121,499 people have died as of October 19th (1). Many countries have been affected, causing immense stress on healthcare systems worldwide. This is the third epidemic caused by a coronavirus, after Severe Acute Respiratory Syndrome (SARS) in 2002 and Middle East Respiratory Syndrome (MERS) in 2012 (2,3). The clinical presentation ranges from asymptomatic or mild disease to severe pneumonia in which the most severe cases deteriorate with acute respiratory distress syndrome (ARDS) requiring prolonged mechanical ventilation, or even Extra-corporeal Membrane Oxygenation (ECMO) (4,5). Approximately 16-35% develop severe pneumonia, 2-17% need mechanical ventilation, of whom up to 15 % need ECMO therapy (6,7,8), and the case fatality rate is 1.4-15% (5,9,10). In the pathophysiology of severe COVID-19, the host immune response plays a key role and it has become evident that COVID-19 pneumonia is associated with both hyper inflammation and immune paralysis (11). A clinical presentation of massive vascular inflammation, disseminated coagulation, shock, and ARDS is frequently triggered (9-11).

Though many therapies aiming at mitigation of the inflammatory response are being evaluated, strong evidence of benefit is lacking. Corticosteroids might have beneficial effects in overcoming both hyperinflammation and ARDS (4,15-17). Furthermore, they could serve as an easily accessible and affordable treatment option. On the other hand, there are known adverse effects of corticosteroid use, such as delayed viral clearance, opportunistic infections and suppression of the hypothalamic-pituitary-adrenal axis.[2,18,19] Earlier studies done in MERS-CoV and SARS-CoV showed delayed viral clearance, opportunistic infections and hyperglycemia (20-22). Therefore, a high number of observational studies and randomized controlled trials (RCT) on corticosteroids for COVID-19 have been initiated and reported, and the signal is a beneficial effect. The RECOVERY trial was the first to report that the use of dexamethasone as opposed to usual care reduced 28-day mortality in patients requiring oxygen therapy or mechanical ventilation (23). And a prospective meta-analysis of seven randomized clinical trials showed that administration of corticosteroids was associated with lower 28-day all-cause mortality (24). And while initially the World Health Organization (WHO) recommended against corticosteroid treatment, as of September 2nd 2020, the WHO recommends systemic corticosteroids rather than no systemic corticosteroids for the treatment of patients with severe and critical COVID-19 (15,25). Also, the Surviving Sepsis Guideline on management of COVID-19 recommends administration of steroids in patients with severe COVID-19 on mechanical ventilation with ARDS, and in patients with COVID-19 and refractory shock (26).

However, the effectiveness and safety of corticosteroids still remain uncertain, because of scarcity of RCTs and inconclusive observational studies, and reliable data to further shed light on the benefit and harm are needed. Therefore, the aim of this systematic review and meta-analysis of observational studies and RCTs was to evaluate the effectiveness and safety of corticosteroids in COVID-19.

METHODS

Data sources and search strategy

A systematic review according to the PRISMA guidelines was conducted (27). The meta-analysis was retrospectively registered under number 38752 at ISRCTN.org. A comprehensive systematic search was conducted for published studies in Medline/ PubMed, Embase, and Web of Science from December 1st 2019 to October 1st 2020. The search strategy consisted of the components “COVID-19”, “intensive care”, and “corticosteroids” (supplement 8.1).

Eligibility

RCTs and observational cohort studies assessing the effect of corticosteroids in COVID-19 were eligible if they met the following inclusion criteria: adult patients (age ≥ 18 years), COVID-19 patients diagnosed by reverse transcriptase polymerase chain reaction (RT-PCR), reporting on outcome measures in relation to corticosteroid treatment, corticosteroids not restricted for type, dose and duration. Studies concerning pregnant women or children, reviews, case series including less than 15 patients and articles that were not available in English were excluded (28).
Definition of primary and secondary outcomes

The primary outcomes were mortality (i.e short-term mortality as defined in the study, including 28-day, 30-day and hospital mortality) and viral clearance (i.e. as defined by the study, based on RT-PCR in respiratory specimens). Secondary outcomes were: need for invasive mechanical ventilation, duration of mechanical ventilation, ventilator free days or other oxygen therapy as reported by the investigators, length of hospital stay (LOS-hospital) and secondary infections. For exact used definitions see supplement 8.2.

Study selection

Suitable studies were selected in two stages. First, six independent reviewers screened all selected titles and abstracts (JvP, JV, EH, KN, PB, SA). If there was consensus that a study was unsuitable for inclusion, it was excluded. Next, the full-text articles were screened independently by two authors and included if both authors agreed. If needed, the article was discussed with the third reviewer until consensus was reached.

Data extraction and quality analysis

After selection, data were extracted by one and checked by a second investigator (JvP, JV, EH, KN, PB). For each study, the author, journal, country, city and hospital in which the study was conducted, date of start of inclusion, study population, study groups, type, dose, route of administration of corticosteroid, median time before corticosteroid initiation, duration of administration, primary and secondary outcomes and adverse events at any time point after admission were extracted in a standardized data extraction form (supplement 8.2).

For each individual study the quality was assessed. For RCTs the Risk of Bias was assessed on six domains (random sequence generation, concealment of allocation, blinding, selective outcome reporting, incomplete outcome data and other) (29,30). The Newcastle Ottawa Scale was used for validity assessment of observational studies (31,32). The NOS score ranges from 0 (low quality) to 9 (high quality) points.

Data analysis and reporting

For the effect of corticosteroids on mortality, a pooled estimate was calculated and graphically summarized in a forest plot. Data from observational studies were analyzed separately from the RCTs, and both separate results and overall combined outcomes were calculated and summarized in the plot. When available, the adjusted odds ratio (OR), relative risk (RR) from the cohort studies were used for pooling to reduce confounding. Since the endpoint (mortality) occurred relative infrequently, the OR will be close to the RR and therefore we decided to pool both RR and OR estimates of the individual studies (33). Furthermore, a pooled estimate was calculated and graphically summarized in a forest plot for need for mechanical ventilation.

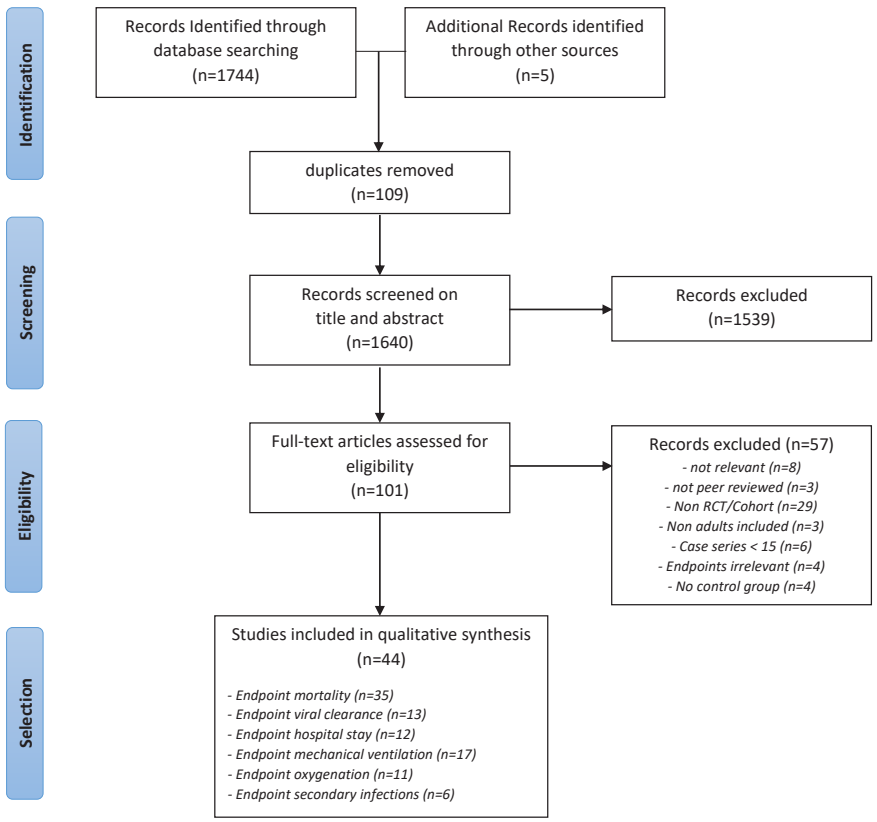
To allow studies to have a different underlying effect, a random effects model was used. I^2 statistics was used to quantify heterogeneity. Furthermore, for the pooled estimate of effect on mortality, tau^2 was used to assess the variance of the true effects. The GRADE approach was used to assess the quality of the evidence for the effect of corticosteroids on mortality. STATA 16.0 was used to perform data analysis.

RESULTS

Study selection

Our search yielded 1640 unique studies. After qualification of title and abstract, 101 studies were selected for full review. Based on exclusion criteria, 57 additional studies were excluded (references in supplement 8.3). The remainder of 44 studies, comprising 20.197 patients, was included in this systematic review and meta-analysis. (Figure 1)

Figure 1. Flowchart Study selection.

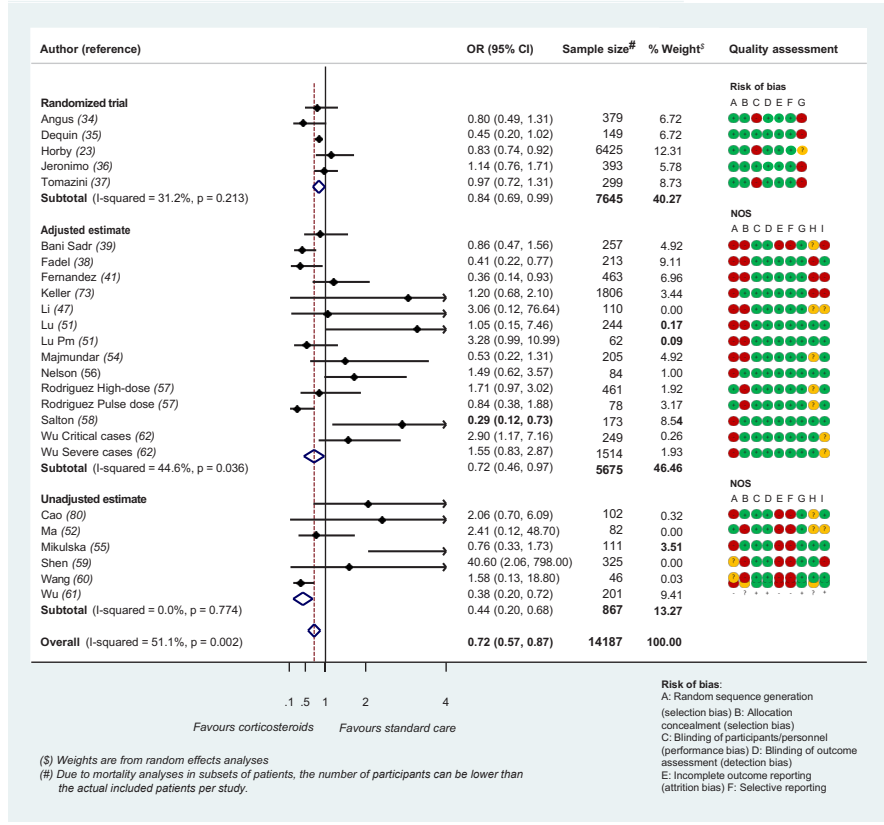


Study Characteristics (table 1 and supplement 8.4)

Thirty-one of the 44 studies originated in China, 11 in Europe, five in North America, two in South America and one study was multi-continental. The inclusion period ranged from late December 2019 until August 20, 2020. The majority of studies were retrospective observational studies (37/44), five were RCTs (23, 34-37), and there were two studies with historical controls (38, 40). The study population varied from hospitalized patients (28/44) to patients admitted to the Intensive Care Unit (ICU) (15/44), and one study included discharged patients for viral clearance assessment. The median age of patients ranged from 34 to 75 years.

For the observational studies the median NOS score was 5 (2-8) points (supplement 8.5). For the RCT the risk of bias table is depicted in Figure 2.

Figure 2. Forest plot mortality.



Corticosteroid Regimen (table 1 and supplement 8.6)

In the 44 studies very diverse corticosteroid strategies were used. If reported (n=35), methylprednisolone was the most frequently prescribed (n = 28) (35,36,38-65). Prednisone (n=5) and dexamethasone (n=5) and hydrocortisone (n=4) were also used, some in studies that allowed multiple corticosteroid regimens (n=9).

Table 1. Study Characteristics

	Author	Reference	Study type	Type - dose [γ] corticosteroids	Sample size	CoVID - Study population	Reporting outcome [α]						Quality score [δ] (Risk of bias or NOS)	Main findings
							M	V	H	R	O	I		
1	**Angus**	**34**	REMAPt [β]	Hydrocortisone < 1 mg/kg ED	403	ICU patients	x		x	x			Risk of Bias[δ]	Two hydorcoritsone dosing resulted high probabilities of superiority with regard to the odds of improvement in organ support–free days within 21 days, compared to standard of care
2	**Bani-Sadr**	**39**	Cohort with historical controls	Prednisolone or Methylprednisolone ≥ 1 mg/kg ED	319	Hospitalized patients	x				x	x	4	Addition of corticosteroids to our institution's COVID-19 treatment protocol was associated with a significant reduction in hospital mortality in the 'after' period
3	**Cao**	**80**	Retrospective Observational	Unknown	102	Hospitalized patients	x						5	Patient characteristics seen more frequently in those who died were development of systemic complications following onset of the illness and the severity of disease requiring admission to the ICU.
4	**Chen Zu**		Retrospective Observational	Unknown	267	Hospitalized patients	x	x					7	Corticosteroid treatment is associated with prolonged viral RNA shedding and should be used with caution.
5	**Chroboczek**	**72**	Retrospective Observational	Unknown	70	Hospitalized patients				x			6	Corticosteroids therapy affected the risk of intubation with a risk difference of −47.1% (95% CI −71.8 to −22.5).
6	**Dequin**	**35**	Randomized controlled trial	Methylprednisolone or Hydrocortisone < 1 mg/kg ED	149	ICU patients with respiratory failure	x			x	x	x	Risk of Bias[δ]	Low dose hydrocortisone, compared with placebo, did not significantly reduce treatment failure (defined as death or persistent respiratory support) at day 21 in critically ill patients.
7	**Fadel**	**38**	Quasi Experimental	Methylprednisolone ≥ 1 mg/kg ED	213	Moderate to severe CoVID patients	x		x	x			6	An early short course of methylprednisolone in patients with moderate to severe COVID-19 reduced escalation of care and improved clinical outcomes
8	**Fang Mei**	**40**	Retrospective Observational	Methylprednisolone < 1 mg/kg ED	78	Hospitalized patients		x					5	Low-dose corticosteroid therapy may not delay viral clearance in patients with COVID-19.
9	**Feng Ling**	**66**	Retrospective Observational	Unknown	476	Hospitalized patients	x		x				5	Differences in AT II receptor inhibitors use were associated with different severities of disease. Multiple lung lobes involvement and pleural effusion were associated with the severity of COVID-19. Advanced age (>75 yr) was a risk factor for mortality.
10	**Fernandez**	**41**	Retrospective Observational	Methylprednisolone ≥ 1 mg/kg ED	463	Patients with ARDS hyperinflammation	x				x		5	Glucocorticoid use is associated with increased survival and improved mortality rates in Severe CoVID-19 patients.
11	**Gazzaruso**	**42**	Retrospective Observational	Methylprednisolone or Prednisone < 1 mg/kg ED	219	Hospitalized patients	x				x		3	Anti-rheumatic drugs, probably steroids included, may modulate inflammation and avoid a hyperinflammation that leads to severe complications and death in subjects with COVID-19.

Table 1. Continued

	Author	Reference	Study type	Type - dose ᵞ corticosteroids	Sample size	CoVID - Study population	Reporting outcome ᵅ						Quality score ᵟ (Risk of bias or NOS)	Main findings
							M	V	H	R	O	I		
12	**Gong Guan**	**43**	Retrospective Observational	Methylprednisolone ≥ 1 mg/kg ED	34	Hospitalized Patients < 50 years		x			x		6	Corticosteroids therapy can effectively release COVID-19 symptoms, improve oxygenation, and prevent disease progression. However, it can prolong the negative conversion of nucleic acids.
13	**Horby**	**23**	Randomized controlled trial	Dexamethasone < 1 mg/kg ED	6425	Hospitalized patients	x		x	x			Risk of Bias[δ]	The use of dexamethasone resulted in lower 28-day mortality among those who were receiving either invasive mechanical ventilation or oxygen alone at randomization but not among those receiving no respiratory support.
15	**Hu Wang**	**44**	Retrospective Observational	Prednisolone or Methylprednisolone ≥ 1 mg/kg ED	308	Hospitalized patients	x	x					4	Glucocorticoid therapy did not significantly influence the outcomes nor the adverse events of COVID-19 pneumonia
16	**Huang Song**	**45**	Retrospective Observational	Methylprednisolone 2 study- groups: High: ≥ 1 mg/kg ED Low: < 1mg/kg ED	64	Hospitalized patients	x						4	There were no significant differences in the duration of severe illness or the number of days on high level respiratory support between low dose and high dose methylprednisolone group. The mean number of days in the hospital was higher in the high dose group
14	**Huang Yang**	**81**	Retrospective Observational	Unknown	60	Severe CoVID patients						x	5	There were no statistically significant differences in immunoglobulin therapy and GCs therapy between the improvement and deterioration subgroups.
17	**Jeronimo**	**36**	Randomized controlled trial	Methylprednisolone < 1 mg/kg ED	393	Hospitalized patients	x		x	x		x	Risk of Bias[δ]	Results showed no overall reduction in mortality in 28 days. Patients over 60 years presented a lower mortality in a subgroup analysis.
18	**Keller**	**73**	Retrospective Observational	Unknown	1806	Early hospitalized patients	x			x			6	In high CRP group, glucocorticoids show significantly reduced risk of mortality or mechanical ventilation (odds ratio, 0.23; 95% CI, 0.08-0.70). In low CRP group glucocorticoids were associated with significantly increased risk of mortality or mechanical ventilation (OR, 2.64; 95% CI,1.39-5.03).
19	**Li Hu**	**46**	Retrospective Observational	Methylprednisolone high and low ED	203	Hospitalized patients		x					5	A dose response relation is suggested for corticosteroids on viral shedding. In addition, high-dose but not low-dose corticosteroids were found to potentially increase mortality in severe patients
20	**Li Li**	**47**	Retrospective Observational	Methylprednisolone or Prednisone < 1 mg/kg ED	475	Non Severe CoVID patients	x	x	x			x	5	Early, low-dose, and short-term corticosteroids therapy was associated with worse clinical outcomes
21	**Li Zhou**	**48**	Retrospective Observational	Methylprednisolone ≥ 1 mg/kg ED	187	Radiologically progressive CoVID patients				x	x		6	Short-term, low-to-moderate-dose corticosteroids benefits patients with LDH levels of less than two times the ULN, who may be in the early phase of excessive inflammation

Table 1. Continued

	Author	Reference	Study type	Type - dose [γ] corticosteroids	Sample size	CoVID - Study population	Reporting outcome [α]						Quality score [δ] (Risk of bias or NOS)	Main findings
							M	V	H	R	O	I		
22	**Lui Fang**	**49**	Retrospective Observational	Methylprednisolone ≥ 1 mg/kg ED	101	Hospitalized patients	x						3	The majority of patients present primarily with fever, and typical manifestations on chest imaging. Middle-aged and elderly patients with underlying comorbidities are susceptible to respiratory failure and may have a poorer prognosis.
23	**Liu Zhang**	**81**	Retrospective Observational	Unknown	1190	Hospitalized patients	x						5	Treatment with glucocorticoids increased the risk of progression from not severe to severe disease (OR 3.79, 95% CI 2.39–6.01)
24	**Liu Zheng**	**50**	Retrospective Observational	Methylprednisolone ≥ 1 mg/kg ED	101	Hospitalized patients		x			x		5	Timely and appropriate application of methylprednisolone in severe and critical patients may improve outcomes and lung function without negative impacts on specific SARS-CoV-2 IgG production
25	**Lu Chen**	**51**	Retrospective Observational	Methylprednisolone, Hydrocortisone or Dexamethasone ≥ 1 mg/kg ED	244	Hospitalized patients	x				x		7	limited effect of corticosteroid therapy could pose to overall survival of critically ill patients with COVID-19. Given the adverse effects, corticosteroid therapy must be commenced with caution, and prudent dosage should be promoted under certain circumstances.
26	**Ma Qi**	**52**	Retrospective Observational	Methylprednisolone 2 study- groups: High: ≥ 1 mg/kg ED Low: < 1mg/kg ED	72	Severe and critical patients	x	x	x	x			6	Corticosteroids cannot reduce the hospital mortality, and is not associated with delayed viral clearance, but it could relieve the inflammatory storm and improve clinical symptoms in brief. Patients with severe COVID-19 could benefit from low-dose corticosteroids.
27	**Ma Zeng**	**53**	Retrospective Observational	Methylprednisolone ≥ 1 mg/kg ED	450	Severe and non-severe patients	x	x	x	x		x	4	Corticosteroids use may be accompanied by increased use of antibiotics, longer hospitalization, and prolonged viral shedding.
28	**Majmundar**	**54**	Retrospective Observational	Prednisolone, Dexamethasone, Methylprednisolone ≥ 1 mg/kg ED	205	Hospitalized patients	x		x	x	x		6	Corticosteroids were associated with a significantly lower risk of the ICU transfer, intubation, or in-hospital death,
29	**Mikulska**	**55**	Retrospective Observational	Methylprednisolone high and low ED	215	Hospitalized non-intubated patients	x				x		6	Early adjunctive treatment with tocilizumab, methylprednisolone or both may improve outcomes in non-intubated patients
30	**Nelson**	**56**	Retrospective Observational	Methylprednisolone ≥ 1 mg/kg ED	117	ICU patients on Mechanical Ventilation	x		x	x			8	Methylprednisolone was associated with increased ventilator-free days and higher probability of extubation in a propensity-score matched cohort.
31	**Rodriquez**	**57**	Retrospective Observational	Methylprednisolone ≥ 1 mg/kg ED	1014	Hospitalized patients	x			x	x		7	Tocilizumab should be prioritized for being tested in randomized trials targeting patients with data suggestive of a hyperinflammatory state The results for PDC were less consistent but are also encouraging.

Table 1. Continued

	Author	Reference	Study type	Type - dose ᵞ corticosteroids	Sample size	CoVID - Study population	Reporting outcome ᵅ						Quality score ᵟ (Risk of bias or NOS)	Main findings
							M	V	H	R	O	I		
32	**Rubio**	**68**	Retrospective Observational	Unknown	92	ICU and General ward patients	x			x			5	The early use of GC pulses could reduce the use tocilizumab and might decrease events such as intubation and death
33	**Salton**	**58**	Retrospective Observational	Methylprednisolone ≥ 1 mg/kg ED	173	ARDS patients	x		x	x			8	Per-protocol administration of prolonged low-dose methylpred-nisolone treatment is associated with a significantly lower hazard of death, reduced ICU burden and decreased ventilator dependence.
34	**Shen Zheng**	**59**	Retrospective Observational	Methylprednisolone unknown dose	325	Hospitalized patients		x					4	COVID-19 cases in Shanghai were imported. Rapid identification, and effective control measures helped to contain the outbreak and prevent community transmission
35	**Shi Wu**	**71**	Retrospective Observational	Unknown	99	Hospitalized patients		x					4	SARS-CoV-2 RNA clearance time was associated with sex, disease severity, and lymphocyte function. The current antiviral protocol and low-to-moderate dosage of corticosteroid had little effect on the duration of viral excretion.
36	**Tomazini**	**37**	Randomized controlled trial	Dexamethasone ≥ 1 mg/kg ED	299	ICU patients with moderate to severe ARDS	x			x		x	Risk of Bias[δ]	Dexamethasone plus standard care compared with standard care alone resulted in a significant increase in the number of ventilator-free days (days alive and free of mechanical ventilation) over 28 days
37	**Wang Jiang**	**60**	Retrospective Observational	Methylprednisolone ≥ 1 mg/kg ED	46	Severe hospitalized patients	x			x	x		7	early, low-dose and short-term application of methylprednisolone was associated with better clinical outcomes in severe CoVID-19 patients, and should be considered before onset of ARDS.
38	**Wang Yang**	**67**	Retrospective Observational	Unknown	69	Hospitalized patients	x						4	COVID-19 shows frequently fever, dry cough, and increase of inflammatory cytokines, and induced a mortality rate of 7.5%. Older patients or those with comorbidities are at higher risk of death.
38	**Wang Zhang**	**69**	Retrospective Observational	Unknown	548	Not Reported	x						6	Low-dose or no glucocorticoid treatment was associated with a lower hazard compared with high-dose treatment (≥ 1 mg/kg) for 15-day in hospital death
40	**Wu Chen**	**61**	Retrospective Observational	Methylprednisolone unknown dose	201	Hospitalized patients	x						4	Treatment with methylprednisolone may be beneficial for patients who develop ARDS.
41	**Wu Huang**	**62**	Retrospective Observational	Methylprednisolone < 1 mg/kg ED	1763	Severe or Critical patients	x						7	Corticosteroid use was not associated with beneficial effect in reducing in-hospital mortality for severe or critical cases in Wuhan.
42	**Xu Chen**	**63**	Retrospective Observational	Methylprednisolone < 1 mg/kg ED	113	Hospitalized patients	x	x					5	Prolonged SARS-CoV-2 RNA shedding was associated with male sex (P = .009), old age (P = .033), concomitant hypertension (P = .009), delayed admission to hospital after illness onset (P = .001), severe illness at admission (P = .049), invasive mechanical ventilation (P = .006), and corticosteroid treatment (P = .025).

Table 1. Continued

	Author	Reference	Study type	Type - dose ᵞ corticosteroids	Sample size	CoVID - Study population	Reporting outcome ᵅ						Quality score ᵟ (Risk of bias or NOS)	Main findings
							M	V	H	R	O	I		
43	**Yang Lipes**	**64**	Retrospective Observational	Methylprednisolone, Hydrocortisone or Dexamethasone ≥ 1 mg/kg ED	15	ICU patients	x				x		6	Possible short-term clinical improvements with corticosteroid. Emphasis the urgent need for high-quality studies on Steroids and outcome in critically ill COVID- 19 patients.
44	**Zha Li**	**65**	Retrospective Observational	Methylprednisolone < 1 mg/kg ED	31	Hospitalized patients	x	x	x				5	No evidence of clinical benefit of corticosteroids was found for those without acute respiratory distress syndrome. Virus clearance may be slower in people with chronic HBV infections.

ᵅ M = mortality; V = Viral Clearnance; H = Length of hospital stay; R = Mechanical Ventilator/respirator; O = Oxygenation; I = Secondary infections.
ᵝ Randomized Embedded Multifactorial Adaptive Platform trial
ᵞ ED = Prednisolone Equivalent Dose
ᵟ Newcastle Ottawa Scale (N.O.S.) for Retrospective observational studies. Risk of Bias (R.O.B.) for Randomized controlled trials: see figure 2.

The indication to start corticosteroids was described in 12 studies (supplement 8.6): in three studies corticosteroids were started at diagnosis/hospital admission (38,41,56). In five studies ICU admission or respiratory deterioration were the indications to start, either randomized according to study protocol (23,34,35,37) or not randomized (38,48,49,60,64).

In 29 studies the dose of corticosteroids was reported: In 16 studies an equivalent dose of > 1 mg/kg prednisolone was used (37-39,41,43,44,48-51,53,54,56-58,64) and in 11 studies a lower equivalent dose than 1 mg/kg prednisolone (23,34-36,40,42,47,52,62,63,65). In two studies a low and high dose group were present (45,46). The duration of therapy varied within a range of 5-10 days, in observational studies frequently dependent on clinical condition of patients.

Effect of steroids on primary and secondary outcomes (table 2, supplement 8.7).
Thirty-five of 44 studies reported on Mortality. Thirteen of these could not be integrated in the meta-analysis due to only overall mortality reporting (n=5) (45,63,64,66,67), or only descriptive reporting (n=8), i.e. of a trend towards better outcome (n=3) (42,68,69), no effect (n=3) (44,49,65), or negative effect on outcome (n=2) (50,52). For the remainder of 22 studies, a pooled estimate was calculated and graphically summarized in a forest plot (figure 2). The mortality reported in these studies was mainly 28-day mortality (11 studies), in 6 studies in-hospital mortality of shorter duration, and in 5 studies unreported (see supplement 8.7). The overall risk estimate (OR) was 0.72 (95%CI 0.57-0.87), suggesting a beneficial effect of steroids use in COVID-19 patients hospitalized with moderate or severe respiratory failure on mortality. Studies were heterogeneous (overall I2 of 51.1%, p= 0.002) with a between-study variance (tau^2) of 0.048. For the subset of RCTs the risk estimate was 0.84 (95%CI 0.72-0.96) and I^2 and tau^2 were 31.2% (p= 0.213) and 0.0096, corresponding to less heterogeneity and less between-study variance.

Thirteen from 44 studies reported on viral clearance, which most frequently was defined as two consecutive negative RT-PCR on nasopharyngeal swabs, or a cycle time value of 40 or more. In the corticosteroid group viral clearance time ranged from 5 to 29 days, in the standard of care group from 8 to 24 days. In nine of 13 studies viral shedding was delayed in the corticosteroid group (40,43,46,47,53,59,63,65,70). In the other four studies, viral clearance was equal (n=2) (50,71), or even better in the corticosteroid group (n=2) (44,52). The numbers are too small to quantify the effect of corticosteroids on viral shedding, or to compare viral shedding time in subgroups of severity of COVID illness, dose, type or timing of corticosteroids administered. (supplement 8.8)

In twelve studies length of hospital stay was compared in both corticosteroid and non-corticosteroid groups. The outcomes varied between studies: six reported longer hospital stay in the corticosteroid group (36,47,53,56,66) and five reported the opposite (23,34,38,52,54) or no effect on hospital stay (58).

Table 2. Summary of findings

Effect of Corticosteroids in hospitalized CoVID-19 patients. *Intervention: Corticosteroids; Comparison: Standard of Care*						
Outcomes	**total nº events/total nº of patients**		**Relative effect (95% CI)**	**Nº of participants (Nº of studies)**	**Certainty of evidence (Grade[α])**	**Comments**
	standard care	**corticosteroids**				
In-hospital Mortality	1547/9080 (17.0%)	1173/5234 (22,4%)	**Estimate 0.72 (0.57 - 0.87)**	14.187[β] (22)	***RCT:*** Moderate ***Non RCT:*** Very low	Corticosteroids reduce mortality in CoVID-19 hospitalized patients
Requirement of Mechanical ventilation	124/467 (26,6%)	89/472 (18,9%)	**Estimate 0.70 (0.54 - 0.91)**	939 (7)	***All studies:*** Very low	17 studies reported on mechanical ventilation, but effects could only be quantified in 7 studies.
Descriptive results: *Data too heterogenous for quantification of effect*						
Viral Clearance	In corticosteroid group viral clearance time ranged from 10 to 29 days in corticosteroids group and from 8 to 24 days in standard of care group			2.556 (13)	*0x RCT 13x retrospective observational study*	Heterogenous outcome reporting. Corticosteroids are associated with a probable delay in viral clearance.
Length of hospital stay	Conflicting results both in favor and against the use of corticosteroids			9.433 (12)	*2x RCT, 10x retrospective observational study*	Effect of corticosteroids on length of hospital stay is uncertain
Mechanical Ventilation	In 14 out of 17 studies, corticosteroids therapy is associated with beneficial effects on ventilator free days, on respiratory failure requiring mechanical ventilation and time on mechanical ventilator			12.114 (17)	*5x RCT, 12x retrospective observational study*	Beneficial effects of corticosteroids on mechanical ventilation different definitions used)
Oxygenation	Outcome reporting in Saturation, p/F ratio and Oxygen demand. Conflicting results in favor and against the use of corticosteroids			3.211 (11)	*1x RCT, 10x retrospective observational study*	Outcome definition too heterogenous to draw conclusions
Secondary infections	In five out of six studies, secondary infections and antibiotic use are increased			2.145 (6)	*3x RCT 3x retrospective observational study*	Corticosteroids are associated with an increase in infectious complications

[α] Details on GRADE score are available in supplement 8.10.

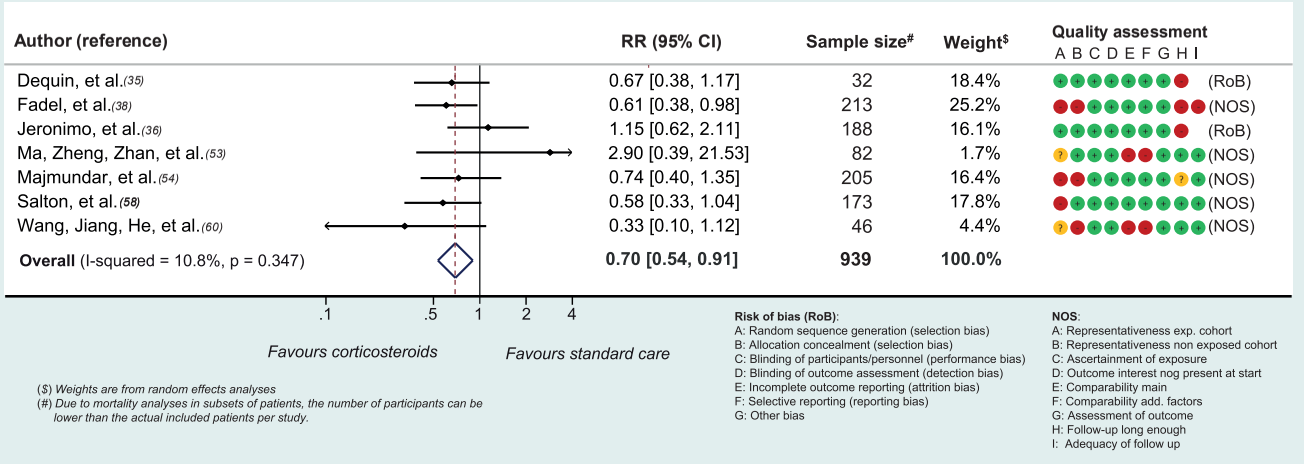
[β] Due to mortality analyses in subsets of patients, this number of participants is lower than the sum of sample sizes from the included study.

Fourteen of 17 studies reported a positive effect of corticosteroids on ventilator free days (34,37,56), on the number of patient requiring mechanical ventilation for respiratory insufficiency (23,35,38,48,54,57,58,60,68,72) or on the time on ventilator (52). In the pooled analyses fewer patients required mechanical ventilation in the corticosteroids group (RR 0.71 (95%CI 0.54-0.97) (Figure 3) though only seven studies supplied sufficient data for this analysis. Jeronimo and Keller failed to demonstrate significant differences (36,73) and one study reported the opposite effect.(53) The dose of corticosteroids could not be related to respiratory outcomes.

Eleven studies reported on the effect of corticosteroids on oxygenation. Various definitions were used: liters per minute of oxygen needed, oxygen saturation, PaO2/FiO2 ratio. The effect of corticosteroids on oxygenation was very heterogeneous: In four studies there was no significant effect (41,42,51,55), in three studies significant improvement was described (50,60,64) and in four studies worse outcome was observed (35,39,54,57).

Six studies addressed secondary infections. More frequently broad spectrum antibiotics were used in de corticosteroid group (39,47,53) and more secondary infections/sepsis episodes were described (35,36). Only Tomazini found a lower percentage of secondary infections in the corticosteroid group. A dose effect of steroids of development of infections/antibiotic need could not be demonstrated.

Figure 3. Forest plot mechanical ventilation.



DISCUSSION

In this systematic review and meta-analysis on effectiveness and safety of corticosteroids in COVID-19 patients, the pooled estimate of the observational retrospective studies and the RCTs supported the positive effect of corticosteroids therapy on mortality in CoVID-19 disease as reported in the RECOVERY trial (23). Furthermore, in already respiratory compromised COVID-19 patients, the need for mechanical ventilation was lower in corticosteroid treated COVID-19 patients. On the other hand, although data in the studies were too sparse to draw any firm conclusions, there might be a signal of delayed viral clearance and an increase in antibiotic use and infections in the corticosteroid group. However, this did associate with prolonged hospital stay or increased mortality.

Besides reviews extrapolating knowledge on SARS-CoV or MERS-CoV (21) or on non-viral ARDS (4), or combining studies on SARS-CoV or MERS-CoV (2,18,74), to our knowledge, only three other meta-analyses on this subject were conducted with conflicting results. (24,75,76) Sarkar et al. found low-quality evidence with high variability that in patients with COVID-19 the steroids may be associated with an around twofold increase in mortality (75). Tlayjeh et al. found no significant difference in mortality or mechanical ventilation need, at the cost of a prolonged viral clearance time (76). The investigators explained that the discordance in studies was due to bias in the large number of non-RCTs. In the third, very robust, prospective meta-analysis of published and pending trials (inclusion has pretty much stopped since the Recovery trial was published), Sterne et al. found that in critically ill patients with COVID-19, administration of systemic corticosteroids, compared with usual care or placebo, was associated with lower 28-day all-cause mortality (24). A downside of this rather robust study was that almost 60 percent of the population consisted of the Recovery study population and a reasonable amount of data was generated from unpublished, unfinished studies.

Compared to these other systematic reviews on corticosteroids and COVID-19, ours was able to include the largest number of studies and COVID-19 patients. Furthermore, we included both observational studies and RCTs to be able to assess adverse effects such as viral clearance and risk of infections. To obtain the highest possible quality, we excluded non-peer reviewed pre-published manuscripts and furthermore, if available, we included adjusted estimates in the meta-analysis, reducing bias by incongruent study groups.

Our review has several limitations. Most of the included studies were retrospective cohort studies with increased risk of bias and lower level of evidence, as we confirmed by the GRADE classification (Table 2, supplement 8.10). Besides that, large heterogeneity in the studies was present (i.e. study population, type, dose, initiation and duration of corticosteroids, outcome measures) and we emphasize that definitions of primary and secondary outcome measures varied greatly and pooled data from this review should be interpreted cautiously. However, we tried to narrow down the outcome measure to short-term mortality. Furthermore, we decided to carefully note the applied definition in the studies in our data extraction tables and include only outcomes as defined by the investigators if they were appropriate for our study, i.e. 28-day or closely related short-term in-hospital mortality. We agree that this variation in definition is indeed a drawback of this review. And although the pooled data from this review should therefore be interpreted cautiously, they represent the effect of corticosteroids on short-term 28-day mortality and the pooled estimates for RCTs, and adjusted and unadjusted observational studies pointed towards the same direction, i.e. of a beneficial effect. In many studies confounding by indication was evidently present: two studies described that corticosteroid administration was “at the discretion of the treating physician” (40,41) and four reported that severe patients were more likely to receive corticosteroid treatment (40,49,60,66) Many studies had incomplete follow-up and a considerable number of patients did not reach definite endpoints. However, our conscious exclusion of non-peer-reviewed studies, the focus on a measurable and quantifiable endpoint, and, if possible, inclusion of risk estimates corrected for confounders and propensity matched, increased the validity of the retrospective evidence supporting the RECOVERY trial. Furthermore, from the included studies, 26 originated in China, with 13 from the hot spot regions (Wuhan, Hubei, Shanghai). This might impair generalizability but although overlapping study populations were present within the included studies (see table in supplement 8.4.), this was only incidentally the case for secondary outcome measures. For the main outcome multiple publication bias was unlikely. (supplement 8.11). Furthermore, 42% of the study population was included from outside China. Moreover, in terms of generalizability, the median age from the included patients in this review ranged from 34 to 72 years. However, data from the CDC state that 42.9% of hospitalized patients in the United States are >65 years and European numbers from the European Centre for Disease Prevention and Control (ECDC) show that 54.2% hospitalized patients are >65 years with great variation between countries (77,78) Despite aforementioned limitations, still, this systematic review and meta-analysis confirms the conclusion of the meta-analysis of the RCTs that critically ill COVID-19 patients hospitalized for moderate or severe respiratory failure, with or without mechanical ventilation, should receive corticosteroids.

Severe COVID-19 patients are faced with a twofold problem. On the one hand, there is the hyperinflammatory response, resulting in pulmonary thrombosis, extravasation of cell debris, and acute lung injury or even ARDS (79). On the other hand there is a need to clear the viral infection itself. This primary phenomenon suggests a possible target for corticosteroids (17). Thus, the confirmation that there is predominantly a beneficial effect of corticosteroids on mortality is congruent with pathophysiological reasoning and prior knowledge. In our study we found a signal of delayed viral clearance, but data in the studies were too sparse to draw any firm conclusions. Therefore, what is lacking is knowledge on the optimal start of corticosteroid administration after the start of illness, specific subpopulations and type, dose and duration. RCTs so far reported a strongly beneficial effect on mortality but did not investigate optimal timing and indication of corticosteroid administration (24). and our study wasn’t able to provide an answer to the latter issues, either. Therefore, future research should focus on which patient characteristics, laboratory and radiological markers can be used to guide indication and timing of corticosteroid treatment, particularly in relation to safety (e.g. delayed viral clearance, increased incidence of secondary infections).

CONCLUSION

In conclusion, our findings from both observational studies and RCTs confirm a beneficial effect of corticosteroids on short-term mortality and a reduction of the need for mechanical ventilation. And although data in the studies were too sparse to draw any firm conclusions, there might be a signal of delayed viral clearance and an increase in secondary infections related to corticosteroid use. Optimal timing, dose and duration of corticosteroids, in relation to safety, remain subject for further investigation. Since corticosteroids are affordable and easily accessible in healthcare systems quivering under the pressure of the global outbreak of this rapidly spreading coronavirus, this field of research should be a universal priority.

ABBREVIATIONS AND ACRONYMS

ARDS:	Acute Respiratory Distress Syndrome
CDC:	Centers for Disease Control and Prevention
CI:	Confidence Interval
COVID-19:	Coronavirus disease 2019
CT:	Computed Tomography
ECDC:	European Centre for Disease Prevention and Control
FiO2:	Inspiratory oxygen fraction
HR:	Hazard Ratio
ICU:	Intensive Care Unit
IQR:	Inter Quartile Range
LOS:	Length Of Stay
MERS-CoV:	Middle East Respiratory Syndrome Corona Virus
OR:	Odds Ratio
NOS:	Newcastle Ottawa Scale
NR:	Not Reported
PaO2:	Arterial oxygen tension
PRISMA:	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RNA:	Ribo Nucleic Acid
RR:	Rate ratio
RT-PCR:	Reverse Transcription Polymerase Chain Reaction
SARS-CoV:	Severe Acute Respiratory Syndrome Corona Virus
SD:	Standard Deviation
Steroids:	Glucocorticoids or Corticoids
SpO2:	Plasma oxygen saturation
WHO:	World Health Organization.

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SUPPLEMENTARY MATERIAL

Contains

1.1	Search strategy
1.2	Data extraction form
1.3	Excluded references
1.4	Data extraction – General information
1.5	NOS score
1.6	Data extraction – Treatment
1.7	Data extraction – Outcome
1.8	Viral Clearance time
1.9	Mechanical ventilation
1.10	Grade classification
1.11	Population bias.

Summary, general discussion and future perspective

SUMMARY OF FINDINGS

This thesis addressed pulmonary complications, i.e. acute lung injury and ARDS, after cardiac surgery and aimed towards better understanding, prediction, and prevention. The thesis was subdivided in three parts and the findings are summarized part by part:

PART 1.

To increase the pathophysiological understanding of the development of acute lung injury after cardiac surgery we studied (a) the alveolar epithelial side of the alveolar-capillary junction and (b) the role of respiratory viruses.

By means of mini bronchoalveolar lavages, we determined inflammatory biomarkers (differential cell counts, IL8 and HNE) in the perioperative time period and we assessed mucociliary clearance by assaying the two major soluble mucins (MUC5AC and MUC5B). We showed a marked increase in the concentrations of MUC5AC and MUC5B in bronchoalveolar fluid of patients after heart surgery and a significant association between the increase of MUC5AC and the severity of lung injury and ICU length of stay. The increase of mucins may simply be one of many reflections of the activation of inflammation that takes place after cardiac surgery, but an etiologic role in the development of lung injury and ARDS after heart surgery cannot be ruled out, especially since the perioperative mucin increase was greater than that of other biomarkers.

To investigate the role of respiratory viruses in the development of lung injury after cardiac surgery we included two studies in this thesis:

- Firstly, we conducted a large cohort study, indirectly investigating the influence of viruses on cardiac surgery using the fact that a patient underwent cardiac surgery in the influenza season as a proxy for a higher frequency of respiratory virus infection. We found a higher frequency of ARDS (9 versus 5%) and a longer duration of mechanical ventilation after cardiac surgery in the influenza season.
- In the second study, we investigated the actual presence of respiratory viruses in miniBAL material of asymptomatic elective cardiac surgery patients. A variety of mostly low virulent respiratory virus RNA, all with high CT-values, was detected, regardless of the season in which patients were operated. A relationship with respiratory outcome could not be established.

PART 2.

To investigate whether biomarkers can predict acute lung injury after cardiac surgery, we studied (a) the inflammatory patterns generated at the endothelial side of the alveolar-capillary junction, and (b) the pre-to-postoperative change of proADM in plasma in relation to development of acute lung injury.

At first, the state of the vascular endothelium side, as part of the alveolar-capillary junction, was further investigated by applying direct flowcytometry to peripheral blood samples taken in the perioperative period from cardiac surgery patients. We demonstrated that, already preoperatively, there was an activated inflammatory system, i.e. higher platelet activatability by means of TRAP and a neutrophil activation pattern with higher expression of both CD11CD18 and CD62L, in the group of patients that developed an acute lung reaction after cardiac surgery. In the same group of patients, we also found a more pronounced inflammatory response in the postoperative time course with increased platelet activation and evident neutrophil activation, i.e. Increase in ligand CD11CD8 and decrease in L-selectin expression CD62. Whether the findings in peripheral blood reflect of cause the status of alveolar endothelium and lung injury remains an important question.

Furthermore, we focused on proADM in plasma, as a multi-potent and biologically active peptide affecting vasomotor status and an important marker of inflammation. To consider the effect of CPB and the surgery itself, a known important factor in the risk of acute lung injury and ARDS, we measured the change of ProADM from pre- to direct post-surgery. This proADM change was a good predictor of the risk of developing ARDS, prolonged duration of mechanical ventilation and increased length of ICU stay. Moreover, the use of this parameter improved the currently used risk model for cardiac surgery, i.e. the EuroSCORE (1).

PART 3.

To investigate whether prophylactic corticosteroids can prevent unfavorable (respiratory) outcomes in cardiac surgery and other ICU patients we conducted three different studies.

In the first retrospective methodological study, the difference in corticosteroid prescribing behavior between anesthesiologists (physicians' preference) was used as an instrument for an instrumental variable analysis (IVA) (2). These findings were compared with an almost simultaneously published RCT on steroid use in cardiac surgery (3). The use of physician's preference as an instrument of IVA was feasible. However, there was no beneficial effect of steroids on cardiac surgical patients in general, and this result was in line with the RCT findings.

Secondly, we conducted a meta-analysis on corticosteroid prophylaxis in cardiac surgery patients, showing similar results: no beneficial effect on mortality could be demonstrated. However, a beneficial effect of steroids was visible on a few important secondary outcome measures: fewer pulmonary complications (including pulmonary oedema, infection or prolonged postoperative ventilation for respiratory failure), less atrial fibrillation and fewer wound infections. The higher incidence of myocardial infarction could not be explained.

The third study comprised a meta-analysis on steroids in a different patient population with ARDS, namely CoVID-19 ICU patients. This study showed a beneficial effect of steroids on duration of ventilation duration, length of ICU stay and mortality. The trend that corticosteroids delay viral clearance and increase opportunistic infections however seems logical but needs further evaluation for their importance in future.

GENERAL DISCUSSION

In this thesis, we come to five main conclusions and additional important remarks:

PART 1. Pathophysiology

1. *Perioperative bronchoalveolar Muc5AC increase was associated with development of acute lung injury and prolonged ICU stay.*

Concentrations of mucins in airway lavage fluid are increased peri-operatively from induction of anesthesia until admission to the ICU, and the increase in MUC5AC is related to the severity of lung injury as measured by P/F ratio. Not only mucins, but in fact all measured biomarkers increased peri-operatively. Thus, the increase of mucins may simply be one of many reflections of the activation of inflammation that takes place after cardiac surgery. However, the magnitude of increase is five- to tenfold for MUC5B and MUC5AC and thereby much more pronounced than the increase of other biomarkers. Additionally, MUC5AC was the only biomarker that was significantly associated with relevant clinical endpoints, such as the severity of lung injury and the length of stay in the ICU. Thus, we cannot rule out that mucins have an etiologic role in the development of lung injury and ARDS after heart surgery.

These findings are in line with other studies. Children with respiratory complications after cardiac surgery showed significantly higher MUC5AC levels than did children without respiratory complications. And the increase of total mucin during cardiopulmonary bypass showed positive correlation with alveolo-arterial oxygen difference (4). Furthermore, in critically ill patients with acute lung injury (ALI), MUC5AC levels in

bronchoalveolar fluid were more than 58-fold increased (5). In normal airways, mucins cover the epithelial surface of the respiratory tract, and mucin production is maintained at a relatively low level to promote mucociliary clearance of inhaled and trapped substances. In pathologic conditions such as asthma, bronchitis, and acute respiratory distress syndrome, however, mucus production is excessive and instead limits mucociliary clearance, and in the small peripheral airways this may lead to mucus plugging and airway obstruction, with ultimately impaired gas exchange. However, the strong association between MUC5AC and lung injury does not necessarily imply that mucins play a direct etiologic role in acute lung injury. Indeed, increased mucin expression could also be just a reflection of the proinflammatory state without a specific causal role.

Our findings must again be put into the perspective of the intrinsic limitations of sampling in the alveolar lavage: (a) Introducing the miniBAL catheter without bronchoscopic guidance leads to an unknown sampling location (6), (b) varying and uncertain dilution factor (7), (c) sampling error: only a limited part of the alveolar space is sampled and it is not clear whether this is representative of the rest of the lung. (6) For these reasons – which as of yet cannot be corrected for - it is difficult to translate findings of sampling in alveolar lavage fluids to the real pulmonary condition.

Another limitation of this study is the fact that BAL is an invasive procedure, performed only while patients were on a mechanical ventilator. A few patients with a good postoperative performance state were already extubated at ICU arrival and were not part of this analysis, which introduces a bias towards the more severe patients. However, the correlation of IL8, HNE, neutrophils, MUC5B and MUC5AC, and the inversed proportional association of MUC5AC with P/F ratio suggest that further research into the inflammatory parameters in the alveolar space could certainly be useful. Finally, the bronchoalveolar lavage is primarily only a reflection of the end product of the mucociliary apparatus: mucus. It would certainly be interesting to also investigate the epithelium of the peripheral lung at the cellular level, to see how other interactants beside mucus i.e. the goblet cells, the club cells, and the pneumocytes, change as acute lung injury and ARDS develops or are changed before.

2. *A contributing role for respiratory viruses in the development of ARDS after cardiac surgery could not be established.*

In our studies, on the one hand, there was an increased development of ARDS and an increased duration of mechanical ventilation during the influenza season, which in fact is only circumstantial evidence that a viral trigger may have played a role in ARDS after cardiac surgery. On the other hand, when measuring the presence of viruses in elective cardiac surgery patients, a relationship with pulmonary complications could not be demonstrated.

The latter rather explorative study, however, showed that asymptomatic elective cardiac surgery patients do carry respiratory viruses both during and outside the influenza season. Almost all of these viruses, however, were low pathogenic and had a low load (high CT values) which might explain their yet non-significant role in development of ARDS (virus group 3/6 and non-virus group 16/43).

Although our studies were inconclusive about the role of respiratory viruses in development of ARDS after cardiac surgery, their role has not been definitively ruled out either. It would still be useful to know how many of the patients who develop ARDS after cardiac surgery actually carry a virus in their respiratory tract. In particular, it would be interesting to learn which specific viruses, and with what CT value, do significantly contribute to the development of ARDS.

PART 2. Prediction

3. *Preoperative increased platelet-activatability and neutrophil activation is related to lung injury. Cell activation patterns in cardiac surgery patients allow us to identify patients that develop lung injury early and may improve preoperative risk models.*

We demonstrated that prior to commencement of cardiac surgery an upregulated inflammatory state is present in patients who develop (acute) lung injury. The observed higher platelet-activatability and signs of a higher neutrophil turn over before surgery, and a typical neutrophil activation pattern later could well contribute to more severe acute lung injury in this respect.

Indeed, one has to be cautious to equate the results of assessments in the peripheral bloodstream with the processes that actually take place at the alveolar-capillary junction. When the vascular endothelium and neutrophils become activated, it initiates a process of tethering, rolling, adhesion, crawling and transmigration (8,9) of the neutrophils. Because of this process, these cells, however, cannot be longer measured in the peripheral blood. Much more invasive research, i.e. taking blood and alveolar samples simultaneously could prove if findings are relevantly associated. Maybe even taking lung biopsies, would be necessary to gain more insight into the local processes at the alveolar-capillary junction and if and how they can be studied by more accessible biomarkers .

Furthermore, the contribution of the CPB circuit cannot be assessed from our results. Platelets and neutrophils namely can also be activated when they come into contact with the synthetic tubing. It is plausible to assume that the process of neutrophil- and platelet activation will take place in the CPB (10), while some of the activated cells

might also be captured in the CPB circuit. Sequential sampling in the in- and outlet cannulas from the circuit would therefore give more insight in the inflammatory role of the CPB circuit.

Hence, whether the higher level of platelet activatability and neutrophil turn-over that we found in patients that developed lung injury, is due to a common (causal) factor or that patients who develop a lung reaction can actually be recognized by such parameters remains difficult to answer. On the other hand, activated inflammation before surgery in this regard likely contributes to the mechanisms we know to play a role in lung injury. Flow cytometry hence could be a valuable addition to current scoring systems and help to better recognize patients who are facing a complicated course.

4. *Development of ARDS post cardiac surgery could be predicted with the pre-to-post surgery increase of proADM. Adding proADM to existing scoring systems, such as the EuroSCORE, ameliorated the predictive capacity.*

By using this two-point biomarker as a predictor of ARDS, it was possible to consider the impact of the surgery itself in addition to the usual risk factors. After all, the duration of the CPB (11,12), and the operative injury itself (12) are both well-known risk factors for the development of ARDS and are reflected by the proADM increase. The use of this biomarker, would make it possible to quantify surgery related risk factors shortly after admittance at the ICU and identify patients with a higher risk for development of ARDS.

Then almost directly the question rises “What can we do with that information?” Unfortunately, except for supportive care, no disease modifying therapy is currently available (13). If causality – in other words if ADM would play a role in later lung injury – our knowledge about the ARDS risk should lead to faster and better deployment of the components of specific therapy influencing this pathway. Till then, supportive therapy, such as lung protective ventilation and stringent fluid policy (14), and probably administration of corticosteroids to prevent worse outcome remain. (this thesis, 15)
Most preferable is a biomarker that discriminates at a timepoint when choices can still be made: before the operation. With a large proADM increase, the damage has already been done and the highest achievable remains damage control in the form of rapid adjustment of good supportive care. Only when better treatment modalities for ARDS are available, this biomarker in sequential samples can play a role in the treatment of ARDS

PART 3. Prevention

5. *The use of corticosteroids does not prevent ARDS or other unfortunate outcomes in cardiac surgery in general, though they are very effective in other ARDS patient groups.*

Meta-analyses (16) and both the DECS and the SIRS trial (3,17) have been performed to study the effect of corticosteroids in cardiac surgery patients. Our studies show similar results: prophylactic steroids are not useful for cardiac surgical patients, in general. On the contrary, steroids are notorious for their side effects on the muscle and nervous system, glucose regulation, and immune system; all factors that one would want to keep optimal in the recovery phase after major surgery.

Even though we are all aware of their side effects, many experts in the field do provide these medicines to a select group of patients, i.e. expert-based personalized medicine. From a pathophysiological point of view, the administration of corticosteroids in a subgroup of patients can certainly be rationalized: there is a group of patients who are at high risk of development of SIRS or ARDS after surgery (i.e. complex surgery, duration of CPB, multiple transfusions, amongst others). In that group, regardless of all these RCTs and meta-analyses, clinicians feel lower thresholds for the use of corticosteroids. Since there was an actual effect on secondary pulmonary outcomes, it could be similar to the history of mechanical ventilation in prone position in ARDS (18-20): the clinician knows the effect from practice and continues to apply it, but the scientific basis lags behind expert observations.

It therefore seems likely that within the total group of cardiac surgical patients, a subgroup can be identified, in which the effect of corticosteroids is valuable. These patients are likely the ones prone for development of an inflammatory respons, i.e. patients undergoing complex interventions and procedures with expected long pump runs (re-operations, complex surgery). In addition, consideration should be given to include the preoperative inflammatory biomarker profile, which was shown (in this thesis) to identify patients prior to their surgery for their (pulmonary) complicated course.

FUTURE PERSPECTIVE

Because the general population is aging (21), health care is becoming more expensive, (22) and accessibility will become an increasing problem due to staff shortages in all branches of health care (23). Therefor, it is more and more important to take steps in the (near) future towards personalized pinpointed medicine. In this regard, with the

advancement of surgical and non-surgical techniques, it is increasingly possible to present patients with a choice of treatment. Examples of this are open heart surgery versus catheter techniques (24, 25), or limitation of interventions to strictly necessary or even refraining from the intervention due to too high risk.

To make these patient- and conditions-specific considerations, it is very important to make a thorough risk assessment before the procedure, so that a patient can be you can inform the individual patient better about his/ her specific risk profile associated with the operation and which alternatives, if any, are available. There are the well-known scoring systems for this risk assessment: EuroSCORE, frailty score, NYHA score, CCS, ASA score, but further optimization and personalization of these scoring systems certainly is desirable.

To make the transition to more personalized medicine, further research is necessary. The directions of research one might think about are:

1. To increase the knowledge of pathophysiology at the alveolar side of the alveolar-capillary junction in patients who develop acute lung injury and ARDS after cardiac surgery and find easily accessible and measurable biomarkers that reflect the junctional condition. Furthermore, gaining more insight at the local level clearly offers better possibilities for (drug) intervention strategies, which reduce postoperative morbidity and mortality and improve the postoperative quality of life.
2. To increase knowledge of pathophysiology at the vascular endothelium side of the alveolar capillary junction. Insight in increased activation and increased turn-over of cells involved in the inflammatory response both increases pathophysiological (etiological) insight as well as prognostic insight. And both can lead to tailored, more patient-centered therapy.
3. To Identify avoidable risk and, with this, enable also avoidance of risk. In relation to this thesis, this would be further exploration of the role of viruses in development of ARDS after cardiac surgery and developing smart vaccination or screening strategies.
4. To improve risk prediction scores with biomarkers, such as proADM. In this regard, it is important that the biomarker provides a good reproduction of the patient's current inflammatory state on top of current scoring systems, which reflect the patient's history and performance state.
5. To expand tailor-made advice and treatment. With the help of the above mentioned more pathophysiological based biomarkers this will become better achievable.
6. An important challenge is to find treatment modalities that limit or inhibit the systemic inflammatory response, the catalyst of ARDS in cardiac surgery patients. The current expert-based personalized medicine with regard to the administration of corticosteroids could be further explored in the high-risk population. In addition, other targeted anti-inflammatory interventions could be investigated in especially the high-risk population.
7. Furthermore, the rapidly emerging innovative technologies could play a role in, for example, the development of more hypo-inflammatory CPB circuits to at least minimize all contributing factors to the development of inflammation.

All the above-described items, and probably many others, can bring us closer towards better understanding, prediction, and prevention of ARDS after cardiac surgery and will contribute to developing the most optimal form of personalized care.

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Samenvatting, algemene discussie en toekomstvisie

SAMENVATTING

In dit proefschrift onderzochten we pulmonale complicaties (Acute Lung Injury (ALI) en Acute Respiratory Distress Syndrome (ARDS)) na hartchirurgie. Drie aspecten werden nader onderzocht: de oorzaak, het voorspellen en het voorkómen van ALI/ARDS na hartchirurigie. De bevindingen zijn hieronder per deelonderwerp samengevat.

DEEL 1. Oorzaken.

Om de pathofysiologie van acute longschade na hartchirurgie beter te begrijpen, onderzochten we (a) de inflammatoire patronen ter plaatse van de alveolaire-capillaire overgang en (b) de rol van respiratoire virussen.

In sequentiële perioperatieve non-fiberscopische mini broncho-alveolaire lavages (miniBAL) werden biomarkers van inflammatie (leukocyten telling en differentiatie, IL8 en HNE) bepaald en de mucociliaire klaring werd onderzocht door bepaling van de twee belangrijkste oplosbare mucines (MUC5AC en MUC5B). Er was een opvallende perioperatieve toename van de MUC5A en MUC5B concentraties in het broncho-alveolaire lavaat van hartchirurgische patiënten. Er was een significantie associatie van de perioperatieve stijging van MUC5AC met zowel de ernst van longschade na de operatie, als de Intensive care opname duur van deze patiënten. De toename van mucines kan eenvoudigweg een reflectie zijn van ontstekingsactivatie die plaatsvindt na hartchirurgie, maar een etiologische rol bij de ontwikkeling van ALI/ARDS na hartchirurgie kan niet worden uitgesloten, vooral omdat de perioperatieve toename van mucines opvallend veel groter was dan die van andere biomarkers.

In dit proefschrift zijn twee studies opgenomen, die als doel hadden de rol van respiratoire virussen bij de ontwikkeling van ALI/ARDS na hartchirurgie te bepalen:

- De eerste is een grote cohortstudie, waarin indirect de invloed van virussen op hartchirurgie werd onderzocht. Hierbij werd de (respiratoire) uitkomst na hartchirurgie onderzocht in relatie tot het seizoen waarin de operatie plaatsvond. Een open hart operatie binnen het griep seizoen werd gebruikt als proxy voor een hogere frequentie van virusinfecties. Bij patiënten die in het griepseizoen werden geopereerd werd een hogere frequentie van ARDS (9 versus 5%) en langere beademingsduur waargenomen ten opzichte van de patiënten die hartchirurgie ondergingen buiten het griepseizoen.
- Daarnaast onderzochten we in de tweede pilot studie de daadwerkelijke aanwezigheid van respiratoire virussen in via miniBAL verkregen luchtwegmateriaal van asymptomatische electieve hartchirurgische patiënten. Een verscheidenheid aan respiratoire virussen, allemaal met hoge CT-waarden (en dus lage load), werd gedetecteerd, ongeacht het seizoen waarin patiënten werden geopereerd. Een relatie met de

respiratoire uitkomst kon niet worden vastgesteld, maar daarvoor was de studie eigenlijk ook te klein.

DEEL 2. Voorspellen.

Uitgaande van ontstekingsacitvatie aan de endothelialse zijde van de alveolaire-capillaire overgang, onderzochten we of biomarkers het ontstaan van ALI/ARDS na hartchirurgie zouden kunnen voorspellen. Enerzijds bestudeerden we activeerbaarheid en activatie van witte bloedcellen en bloedplaatjes aan de endotheliale zijde van de alveolaire-capillaire overgang en anderzijds onderzochten we de perioperatieve stijging van proADM in relatie tot de ontwikkeling ALI/ARDS, uitgedrukt in de P/F ratio.

Voor het onderzoek naar activatie en activeerbaarheid van de leukocyten en trombocyten werd op sequentiële tijdstippen bij hartchirurgische patiënten bloed afgenomen en onderzocht op cellulaire activatie door middel van directe flowcytometrie. In de groep patiënten die na hartchirurgie een ALI/ARDS ontwikkelden, toonden we aan dat er preoperatief, nog voor de operatie überhaupt was begonnen, sprake was van geactiveerde witte bloedcellen en bloedplaatjes: de activeerbaarheid van trombocyten door thrombin receptor activating peptide (TRAP) was significant hoger en er was sprake geactiveerde neutrofielen, blijkend uit de toegenomen expressie van zowel ligand CD11CD18 als L-selectine CD62. In dezelfde groep patiënten vonden we ook een meer uitgesproken ontstekingsreactie in het postoperatieve tijdsverloop met verhoogde activering van bloedplaatjes en een duidelijk activatiepatroon van de neutrofielen met toename in expressie van ligand CD11CD8 en afname van L-selectine CD62.

Verder onderzochten we de voorspellende waarde van proadrenomedulline (ProADM). ProADM is een multi-potent en biologisch actief peptide dat de vasomotorische status beïnvloedt en daarnaast is het een belangrijke marker van ontsteking. Om ook het effect van de hart-long machine en de operatie zelf mee te kunnen wegen als bekende risicofactoren van ARDS, hebben we de proADM verandering van pre-naar post-operatief gemeten en gerelateerd aan de ontwikkeling van ARDS en andere klinische parameters. Deze perioperatieve proADM stijging bleek een goede voorspeller van het risico op het ontwikkelen van ARDS, verlengde duur van mechanische beademing en langer verblijf op de IC. Bovendien verbeterde het gebruik van deze parameter de frequent gebruikte risicoscore binnen de hartchirurgie; de EuroSCORE.

DEEL 3. Voorkómen

Om de effecten van profylactische corticosteroïden op de (respiratoire) uitkomsten bij hartchirurgie te onderzoeken, hebben we drie verschillende onderzoeken uitgevoerd.

In de eerste retrospectieve, maar ook methodologische studie is het verschil in voorschrijfgedrag van corticosteroïden tussen anesthesiologen (de voorkeur van de arts) gebruikt als instrument binnen een instrumentele variabele analyse (IVA). De bevindingen van dit onderzoek werden vergeleken met de bijna gelijktijdig gepubliceerde randomized controlled trial over steroïdengebruik bij hartchirurgie, de DECS studie. Het gebruik van de voorkeur van de arts als instrument van IVA was haalbaar. Daarnaast was er geen gunstig effect van steroïden op cardiale chirurgische patiënten in het algemeen, en dit resultaat was complementair met de bevindingen van de DECS trial.

De tweede studie is een meta-analyse naar corticosteroïdprofylaxe bij hartchirurgische patiënten, die vergelijkbare resultaten liet zien: er kon geen gunstig effect van steroiden op mortaliteit worden aangetoond. Wel was er een gunstig effect van steroïden zichtbaar op enkele belangrijke secundaire uitkomstmaten: minder pulmonale complicaties (waaronder longoedeem, infectie of langdurige postoperatieve beademing bij respiratoire insufficiëntie), minder boezemfibrilleren en minder wondinfecties. De hogere incidentie van myocardinfarct kon niet worden verklaard.

De derde studie omvatte een meta-analyse van steroïden in een andere patiënten-populatie met ARDS, namelijk CoVID-19 IC-patiënten. Deze studie toonde wel een gunstig effect aan van steroïden op beademingsduur, ligduur op de IC en mortaliteit.

ALGEMENE DISCUSSIE

In dit proefschrift komen we tot vijf hoofdconclusies, waarbij we een aantal opmerkingen en belangrijke kanttekeningen moeten plaatsen:

DEEL 1. Pathofysiologie

1. *Perioperatieve broncho-alveolaire Muc5AC stijging is geassocieerd met de ontwikkeling van acute longschade en verlengde ligduur op de IC.*

De perioperatieve (van het moment direct na inductie tot het moment van binnenkomst op de IC) verandering van de mucine concentratie in miniBAL vloeistof van hartchirurgie patiënten die een ALI/ARDS ontwikkelen is verhoogd en de perioperatieve toename in MUC5AC is gerelateerd aan de ernst van longletsel zoals gemeten met de P/F-ratio. Niet alleen mucines, maar feitelijk alle gemeten alveolaire biomarkers lieten een perioperatieve stijging zien. Dus, de toename van mucines kan eenvoudigweg een van de vele reflecties zijn van een inflammatoire respons na hartchirurgie. Echter, de omvang van de toename (vijf- tot tien keer) voor MUC5B en MUC5AC was veel groter

dan de toename van andere biomarkers. Opvallend is dat MUC5AC de enige biomarker was die significant geassocieerd was met relevante klinische eindpunten, zoals de ernst van longletsel en de duur van verblijf op de ICU. Aldus kunnen we niet uitsluiten dat de vorming van mucines een etiologische rol speelt bij de ontwikkeling van longletsel en ARDS na hartchirurgie.

Deze bevindingen komen overeen met andere studies. Kinderen met respiratoire complicaties vertoonden significant hogere MUC5AC-niveaus dan kinderen zonder respiratoire complicaties en er was een positieve correlatie tussen de mucine toename en de alveolaire-arteriële zuurstof verschillen. Daarnaast was het broncho-alveolaire MUC5AC niveau bij kritisch zieke IC patiënten met acute longschade (ALI) meer dan 58 keer verhoogd. In normale luchtwegen bedekt een dun laagje mucines het epitheliale oppervlak van het ademhalingssysteem, en de mucine productie wordt gehandhaafd op een relatief laag niveau om mucociliaire klaring van ingeademde en vastgehouden stoffen te bevorderen. In pathologische omstandigheden, zoals astma, bronchitis en ARDS, beperkt overmatige mucusproductie echter de mucociliaire klaring, terwijl mucusophoping in de kleine perifere luchtwegen kan leiden tot mucus propvorming en luchtwegobstructie, waardoor uiteindelijk de gaswisseling verstoord raakt. De sterke associatie tussen MUC5AC en de ontwikkeling van ALI/ARDS betekent echter niet dat mucines een direct etiologische rol spelen. En alternatieve verklaring is dat een toegenomen mucine-expressie een reflectie is van de pro-inflammatoire toestand zonder dat er een specifieke causaal verband is.

Er zijn een aantal kanttekeningen, inherent aan monsterafnames uit de broncho-alveolaire ruimte, te plaatsen bij onze bevindingen: (a) Het “blind” (zonder bonchoscoop) inbrengen van de miniBAL-katheter geeft onduidelijkheid over de precieze locatie waar het monster vandaan komt, (b) er is een variabele en onzekere verdunningsfactor, (c) slechts een beperkt deel van de alveolaire ruimte wordt bemonsterd en het is niet duidelijk of dit representatief is voor de rest van de long. Het is moeilijk om adequaat te corrigeren voor deze intrinsieke beperkingen van monsterafname in alveolaire lava-gevloeistoffen.

Een andere beperking van deze studie is het feit dat een invasieve BAL alleen werd uitgevoerd terwijl patiënten beademd werden. Een paar patiënten met een goede postoperatieve toestand werd direct postoperatief op de operakamers al geëxtubeerd en waren uitgesloten van postoperatieve monstername, wat de resultaten kan hebben beïnvloed.

Al met al, suggereert de correlatie van IL8, HNE, neutrofielen, MUC5B en MUC5AC, en de omgekeerde proportionele associatie van MUC5AC met de P/F-ratio, dat verder onderzoek naar de inflammatoire parameters in de alveolaire ruimte nuttig zou kunnen zijn. Bovendien is de broncho-alveolaire lavage primair slechts een reflectie van het

eindproduct van het mucociliaire apparaat: mucus. Het zou ook interessant zijn om ook het alveolaire epitheel op cellulair niveau te onderzoeken, om te zien hoe andere factoren dan mucus zelf, zoals de slijmbekercellen, de clubcellen en de pneumocyten, veranderen naarmate acute longbeschadiging en ARDS zich ontwikkelen.

2. Een bijdragende rol van respiratoire virussen in de ontwikkeling van ARDS na hartchirurgie kon niet worden vastgesteld.

In onze studies was er enerzijds een toegenomen ontwikkeling van ARDS en een langere duur van mechanische ventilatie tijdens het griepseizoen, wat in feite slechts indirect bewijs is dat een virale trigger mogelijk een rol heeft gespeeld bij ARDS na hartchirurgie. Anderzijds kon bij het meten van de aanwezigheid van virussen bij patiënten die een electieve hartoperatie ondergingen, geen verband met pulmonale complicaties worden aangetoond.

De laatste, eerder verkennende studie toonde echter aan dat asymptomatische patiënten die een electieve hartoperatie ondergingen, zowel tijdens als buiten het griepseizoen respiratoire virussen bij zich dragen. Bijna al deze virussen waren echter laag-pathogeen en hadden een lage viral load (hoge CT-waarden), wat hun niet-significante rol in de ontwikkeling van ARDS zou kunnen verklaren (virusgroep 3/6 en niet-virusgroep 16/43).

Hoewel onze studies niet doorslaggevend waren over de rol van respiratoire virussen in de ontwikkeling van ARDS na hartchirurgie, is hun rol ook niet definitief uitgesloten. Het zou nog steeds nuttig zijn om te weten hoeveel van de patiënten die ARDS ontwikkelen na hartchirurgie daadwerkelijk een virus in hun luchtwegen dragen. In het bijzonder zou het interessant zijn om te weten welke specifieke virussen, en met welke CT-waarde, significant bijdragen aan de ontwikkeling van ARDS.

DEEL 2. Voorspellen

3. Hogere preoperatieve waarden van bloedplaatjes- en neutrofielen activatie zijn gerelateerd aan de ontwikkeling van longschade na hartchirurgie.

Cel activatie patronen kunnen helpen bij vroege identificatie van patiënten at risk voor het ontwikkelen van een ALI/ARDS en zouden bestaande preoperatieve risicomodellen kunnen verbeteren.

We hebben aangetoond dat, zelfs voordat de operatie is begonnen, een hoger activatie-patroon van bloedplaatjes en neutrofielen aanwezig was in de patiëntengroep die uiteindelijk een ALI/ARDS ontwikkelde. Bovendien hadden patiënten die een acute longreactie ontwikkelden ook een toegenomen activatie van bloedplaatjes en een verhoogde neutrofielenturnover tot 24 uur na het begin van de operatie.

De resultaten van de metingen in het perifere bloed moeten met enige terughoudendheid geïnterpreteerd worden. Immers, wat gemeten kan worden in het perifere bloed, kan niet zomaar gelijk gesteld worden aan de processen die feitelijk plaatsvinden op de alveolaire-capillaire overgang. Bij activatie van neutrofielen en het vasculaire endotheel komt er bij de neutrofielen en trombocyten een proces op gang van rollen, binden, kruipen en transmigratie. Echter zodra geactiveerde neutrofielen en trombocyten binden aan het endotheel, zijn ze niet meer meetbaar in het perifere bloed. Veel ingrijpender onderzoek, namelijk het gelijktijdig afnemen van bloed en alveolaire monsters en misschien zelfs het nemen van longbiopten, zou nodig zijn om meer inzicht te krijgen in de lokale processen op de alveolair-capillaire overgang.

Verder is niet uit te sluiten dan het gebruik van de hartlongmachine onze resultaten heeft beïnvloed. Bloedplaatjes en neutrofielen raken geactiveerd bij contact met de kunstmaterialen van de machine. En het kan niet worden uitgesloten dat sommige van de geactiveerde cellen juist in de hartlongmachine worden weggevangen. Sequentiële bemonstering in de in- en outlet canules kan meer in inzicht geven in rol van de hartlongmachine bij de inflammatoire respons op hartchirurgie en de ontwikkeling van longschade.

Een belangrijke bevinding was het feit dat er al preoperatief een hoger niveau van trombocytenactivatie en een grotere neutrofielenturnover aanwezig was bij patiënten die een ALI/ARDS ontwikkelden na hartchirurgie. Het blijft moeilijk, maar daarom niet minder belangrijk, om te onderscheiden of hier een gemeenschappelijke (causale) factor speelt of dat patiënten die een longreactie ontwikkelen, daadwerkelijk al preoperatief kunnen worden herkend aan dit inflammatoire profiel. Het is zeer wel mogelijk dat peroperatieve ontstekingsactivatie bijdraagt aan de ontwikkeling van longschade na hartchirurgie, naast de andere bekende factoren. Flowcytometrie kan daarom een waardevolle aanvulling zijn op de huidige score- systemen en helpen om patiënten te herkennen, die een gecompliceerd postoperatief beloop tegemoet gaan.

4. *De ontwikkeling van ARDS na hartchirurgie kon worden voorspeld met de pre- naar-postoperatieve toename van proADM. Toevoegen van deze biomarker aan bekende scoresystemen, zoals de EuroSCORE, verbeterde ook de voorspellende waarde van deze score.*

Door het gebruik van een twee-punts biomarker als voorspeller van ARDS, was het mogelijk om naast preoperatieve risicofactoren ook rekening te houden met de impact van de operatie zelf. Immers, de tijd aan een hartlongmachine en de weefselschade door operatie zelf zijn ook bekende risicofactoren voor de ontwikkeling van ARDS. Met het gebruik van deze perioperatieve biomarker is het mogelijk om de door de operatie geïnduceerde inflammatoire respons al kort na de IC opname te kwantificeren en

patiënten te identificeren met een hoger risico op het ontwikkelen van ALI/ARDS. Blijft wel de vraag hoe deze informatie het klinisch beloop kan beïnvloeden. Er is namelijk, behalve ondersteunende zorg, momenteel geen behandeling waarmee ARDS kan worden voorkomen of genezen. Het al bij binnenkomst op de IC kwantificeren van het risico op ALI/ARDS, zal vooral leiden tot snellere initiatie van maximale supportive care, zoals long protectieve beademing, restrictief vochtbeleid, en eventueel toedienen van corticosteroïden om slechtere uitkomst te voorkomen.

Bij het gebruik van een biomarker, gaat de voorkeur uit naar een biomarker waarmee nog keuzes kunnen worden beïnvloed, wat in de huidige gezondheidszorg neerkomt op een preoperatieve discriminerende biomarker. Immers, bij een forse perioperatieve toename van proADM is de ARDS al in ontwikkeling en blijft het hoogst haalbare schadebeperking door het leveren van adequate supportive care. Alleen als er betere behandelmodaliteiten voor ARDS beschikbaar komen, kan een biomarker in sequentiële monsters een rol spelen bij de behandeling van hartchirurgische patiënten.

DEEL 3. Voorkómen

5. *Hoewel corticosteroïden effectief waren bij de behandeling van ARDS ten gevolge van CoVID-19, kon het gebruik van corticosteroïden ARDS of andere ongunstige uitkomsten bij hartchirurgie in het algemeen niet voorkomen.*

In aanvulling op de DECS- en SIRS-trials en eerdere meta-analyses, werd in dit proefschrift het effect van corticosteroïden op uitkomsten bij hartchirurgische patiënten bestudeerd met vergelijkbare resultaten: profylactische steroïden zijn over het algemeen niet nuttig voor hartchirurgische patiënten. Integendeel, ze hebben notoir ongunstige bijwerkingen op het spier- en zenuwstelsel, de glucoseregulatie en het immuunsysteem; allemaal factoren die je optimaal wilt houden in de herstelfase na een grote operatie.

Ondanks deze kennis, worden steroïden door experts in het veld nog steeds toegediend aan een selecte groep patiënten als onderdeel van ‘expert-opinion-based’ gepersonaliseerde geneeskunde. Pathofysiologisch gezien is het selectief toedienen van corticosteroïden wel degelijk te rationaliseren; er is immers een selecte groep patiënten met een verhoogd risico op het ontwikkelen van SIRS en secundaire ARDS na een operatie (dit proefschrift). Daarnaast werd een gunstig effect van steroiden op secundaire respiratoire uitkomstmaten aangetoond (dit proefschrift). Voor die selecte groep patiënten zal de behandelaar, ongeacht de uitkomsten van de gerandomiseerde studies en meta-analyses, een lagere drempel ervaren voor het gebruik van corticosteroïden. Bij het gebruik van steroïden binnen de hartchirurgie zou het net zo kunnen gaan als bij buikligging bij ARDS patiënten: de clinicus ziet en weet uit de praktijk dat er

wel degelijk effect is en blijft ze toepassen; de wetenschappelijke basis loopt slechts achter op de praktijk.

Er kunnen binnen de totale groep hartchirurgische patiënten subgroepen worden onderscheiden, waarin verder onderzoek naar het effect van corticosteroïden waardevol is. Te denken valt aan patiënten die zeer complexe ingrepen ondergaan en patiënten met een verwachte lange pomprun. Daarnaast kan zeker overwogen worden om het preoperatieve inflammatoire biomarkerprofiel een rol te laten spelen in de afwegingen, immers hiermee is het mogelijk hiermee op voorhand patiënten te identificeren met een hoog risico op een gecompliceerd (pulmonaal) beloop.

TOEKOMSTVISIE

Doordat de algemene bevolking vergrijst, de zorg duurder wordt, en beschikbaarheid een steeds groter probleem wordt door personeelstekort in alle takken van de zorg, wordt het steeds belangrijker om (in de nabije toekomst) stappen te zetten richting gepersonaliseerde geneeskunde. Met de vooruitgang van chirurgische en niet-chirurgische technieken is het immers in toenemende mate mogelijk om patiënten een behandelingskeuze voor te leggen. Voorbeelden hiervan zijn open hartchirurgie versus kathetertechnieken of beperken van ingrepen tot het strikt noodzakelijke of zelfs afzien van de ingreep wegens te hoog risico.

Voor het optimaal afwegen van de mogelijkheden, is het van belang om voorafgaand aan de ingreep een gedegen risico-inschatting te maken, zodat de individuele patiënt nog beter geïnformeerd kan worden over het individuele risicoprofiel bij de operatie en over eventuele alternatieve behandelmodaliteiten. Optimalisatie van de huidige scoresystemen (EuroSCORE, frailty-score, NYHA-score, CCS, ASA-score) door uitbreiding met een inflammatoir profiel lijkt hiervoor zeker mogelijk en wenselijk.
Echter voor de overstap naar meer gepersonaliseerde geneeskunde gemaakt kan worden, is verder onderzoek wenselijk. De onderzoeksrichtingen waaraan men zou kunnen denken zijn:

1. Vergroten van kennis over pathofysiologie op de alveolaire-capillaire overgang bij ALI/ARDS na hartchirurgie. Meer inzicht op lokaal niveau biedt mogelijkheden voor het ontwikkelen van (medicamenteuze) interventiestrategieën, die postoperatieve morbiditeit en mortaliteit verminderen en de postoperatieve kwaliteit van leven verbeteren
2. Vergroten de pathofysiologische kennis van de vasculaire endotheelzijde van de alveolaire-capillaire overgang bij ALI/ARDS na hartchirurgie. Kennis over inflammatoir geïnduceerde verhoogde activatie en verhoogde turn-over van cellen vergroot zowel het pathofysiologische (etiologische) inzicht als het prognostische inzicht. En beide kunnen leiden tot personalized therapie.
3. Inventariseren, dan wel mitigeren van vermijdbare risico's. In dit verband kan worden gedacht aan een verdere verkenning van de rol van de verschillende respiratoire virussen bij de ontwikkeling van ARDS na hartchirurgie en het ontwikkelen van vaccinatie- of screeningstrategieën.
4. Optimaliseren van advies en behandeling op maat door het implementeren van biomarkers in de momenteel gebruikte risicomodellen. Hierbij is het belangrijk dat de biomarker een goede weergave geeft van de inflammatoire status praesens van de patiënt. Dit kan de huidige scoresystemen verbeteren, die nu vooral gebaseerd zijn op de medische voorgeschiedenis en actuele performance state van de patiënt.
5. Uitbreiding van advies en behandeling op maat. Met behulp van de hierboven genoemde meer pathofysiologisch gebaseerde biomarkers zal dit beter mogelijk worden
6. De laatste uitdaging is het vinden van behandelingsmodaliteiten die de systemische ontstekingsreactie, de katalysator van ARDS bij hartchirurgische patiënten, beperken of remmen. Zowel de huidige op expert-opinie gebaseerde toediening van corticosteroïden, als ook andere gerichte anti-inflammatoire interventies, zouden met name in de hoog risico populatie verder kunnen worden onderzocht.
7. Bovendien zou de snel opkomende technische geneeskunde, met hun eigen specifieke kijk op zaken, een rol kunnen spelen bij de ontwikkeling van meer hypo-inflammatoire hartlongmachine-circuits om op die manier alle factoren die bijdragen aan de ontwikkeling van ontstekingen te minimaliseren.

Alle hierboven beschreven onderzoeksrichtingen, en waarschijnlijk nog vele andere, kunnen ons een stap dichterbij brengen naar het begrijpen, voorspellen en voorkomen van ARDS na hartchirurgie en zullen bijdragen aan de ontwikkeling van de meest optimale vorm van zorg voor de patiënt als individu.

Words form the author

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CURRICULUM VITAE

Judith van Paassen werd geboren op 3 september 1976 te Lochem, in de achterhoek. Haar peutertijd bracht zij door op een tropisch eiland in het Caraïbisch gebied, Tobago, en daarna woonde zij achtereenvolgens in Lochem en Bemmel. Na het behalen van het VWO diploma aan het Canisius College Mater Dei te Nijmegen, begon zij In 1994 aan de studie geneeskunde aan de Katholieke Universiteit van Nijmegen. Na een laatste co-schap in Ghana behaalde zij haar bul in 2001. Van 2002-2008 was zij in opleiding tot internist; eerst in het Alrijne Ziekenhuis te Leiderdorp en later in het LUMC te Leiden. In 2005 begon zij met haar stage klinische epidemiologie en zette zij haar eerste schreden op het onderzoekspad dat tot dit proefschrift heeft geleid. Na haar registratie als internist, deed zij aansluitend, ook in het LUMC, haar fellowship op de IC onder opleider M.S. Arbous. Sinds 2009 is zij werkzaam als internist-intensivist in het LUMC. Haar aandachtgebieden zijn infecties, infectie preventie en antibiotisch beleid en daartoe heeft zij zitting in diverse LUMC brede commissies. Belangrijke onderdelen van haar werk zijn, naast de kliniek, het opzetten van de zorg rondom VHK (virale hemorragische koorts) patiënten en ze had een belangrijke rol in het (mede) organiseren van de IC zorg rondom de Covid pandemie geweest. Momenteel is zij plaatsvervangend opleider op de IC en daarnaast richt zij zich op taken binnen de beroepsvereniging, de NVIC. Zij is lid van de taskforce acute infectiologische bedreigingen en ze heeft zitting in de stuurgroep van de richtlijnen commissie.

Samen met haar levenspartner Rob de Best heeft ze twee zoons, Ivar en Olav.

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De basis van dit proefschrift ligt in mijn stage bij de afdeling klinische epidemiologie, zoals die door Hans Romijn en Jan Vandenbroucke is ingesteld binnen de opleiding interne geneeskunde. Voor een IC gerelateerd onderwerp klopte ik, zoals velen voor mij (en na mij) al hebben gedaan, aan bij Sesmu Arbous. We kwamen op “steroïden en hartchirurgie”. Ik bouwde een database en ik las alle literatuur die er was over dit onderwerp. Dit resulteerde uiteindelijk in een Cochrane meta-analyse en we zetten de basis voor hoofdstuk 7 van dit boek. Geheel onverwacht, aangezien ik mezelf niet als primaire onderzoeker zag. De epidemiologen bleven hierna altijd laagdrempelig bereikbaar, waarbij ik vooral Olaf Dekkers en Saskia Le Sessie bij naam wil noemen.

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- Geert Groeneveld, Maaike van der Swet en Jutte de Vries: Wat een leuke en interessante spin off was ons virus project en met welk een bijzonder eindresultaat.
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Als laatste wil ik “mijn Rob” bedanken .... -woorden schieten tekort- ... voor alles.

"The substance of the lung is dilatable and extensible like the tinder made from a fungus.
But it is spongy and if you press it, it yields to the force which compresses it,
and if the force is removed, it increases again to its original size"

L. DA Vinci 1507