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

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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)

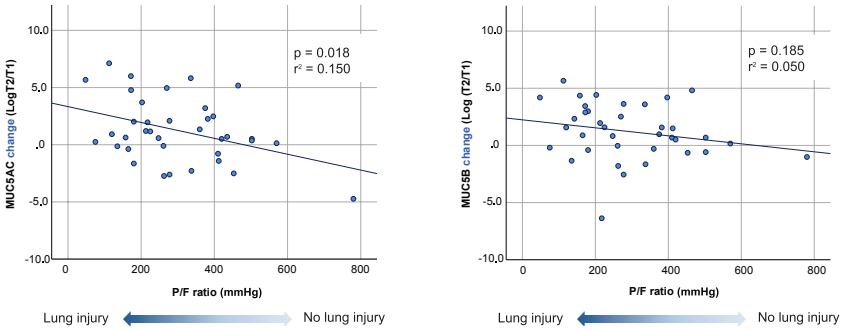
Table 3. Correlation between increase of biomarkers measured in miniBAL as concentration at T2 divided by concentration at T1 (T2/T1), and clinical outcomes.

	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)

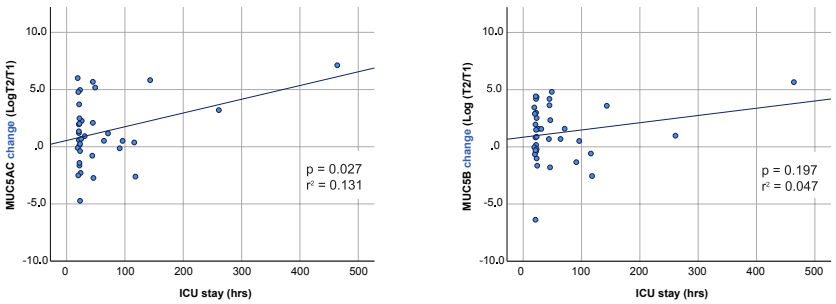
*Correlation with CPB-time, mechanical ventilation time, P/F ratio and ICU-LOS by Pearsons correlation coefficient. Correlation with type of surgery by ETA square correlation statistics. IL-8 = Interleukin 8; HNE = Human Neutrophil Elastase; MUC5B = Mucin 5B; MUC5AC = Mucin 5AC. CPB = cardiopulmonary 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: