

Peri-Operative Changes of Immune Sensitivity in  
Patients Undergoing Cardiac Surgery with  
Cardiopulmonary Bypass or Interventional Cardiology  
Procedures.

**PhD short thesis**

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Budapest,

2015

## Introduction

The systemic inflammatory response syndrome (SIRS) is a serious, life-threatening clinical condition. The prevalence of SIRS is high, affecting one-third of all hospitalized patients.

In cardiac surgery, pre-existing disorders (e.g., poor left ventricular function, diabetes) and surgical factors (e.g., sternotomy, pericardectomy or cardiac ischemia) can cause a pathological inflammatory response and concomitant organ dysfunction. In addition, the extra-corporeal circulation (ECC) is considered as a cause of SIRS. The contact of blood with air and the foreign surfaces included in the ECC circuit triggers a complex cascade of reactions that involve the inflammatory, clotting and complement systems. The coincidence of potential confounders (e.g., advanced age, pre-operative health condition, perfusion technique) may further act unfavorably, which can lead to a dysbalance of pro- and anti-inflammatory pathways and the subsequent development of SIRS.

Interventional cardiology procedures include transcatheter aortic valve implantation (TAVI) via the trans-

femoral (TF-TAVI) or the transapical route (TA-TAVI). Although in TAVI an ECC is avoided, SIRS remains a common phenomenon. SIRS in TAVI occurs in 40-60 % of affected individuals and is independently associated with worse short-term outcome and mortality. In contrast to the mechanisms in cardiac surgery, SIRS pathogenesis and triggering factors have not been completely identified in TAVI. Currently, intra-procedural low cardiac output states are considered partially accountable for the induction of ischemic organ injury and for SIRS.

### Objectives

The aim of this doctoral thesis is to provide a detailed insight into the inflammatory response and the changes in immune sensitivity that occur in the peri-operative period during different cardiac surgery and interventional cardiology procedures.

Study 1. Assessment of the immune sensitivity in cardio-pulmonary bypass procedures using CD62L shedding assay. The aim of was to investigate changes in the peri-operative immune sensitivity in patients scheduled to undergo cardiac surgery by using conventional extra-corporeal circulation (CECC). The main focus was on CECC-induced immunomodulation, which was determined by the quantification of molecules that are involved in immune reactions (i.e., interleukins). In addition, the functional status of the immune system was determined by using a novel method to assess the responsiveness of inflammatory effector cells. This method, referred to as the CD62L shedding assay, quantifies the cleavage of the adhesion molecule L-selectin (CD62L) from the surface of neutrophils after microbial stimulation, thereby providing information on granulocyte and monocyte sensitivity.

Study 2. Investigation of inflammatory response to treatment of AS. The aim was to investigate patients with severe symptomatic aortic valve stenosis (AS) for the inflammatory response in the perioperative course. The main focus lay in the comparison of cardiac surgical and interventional cardiology modalities in the treatment of

AS. Whereas surgical aortic valve replacement (SAVR) using CECC represents the current gold standard, TAVI become widely available for selected high-risk patients with multiple co-morbidities allowing the treatment of AS without the use of ECC.

Study 3. Investigation of inflammatory response to surgical treatment of AS. This study investigated the inflammatory response occurred in patients treated solely surgically for AS. The main focus lay on the comparison of CECC with its less invasive variant, the minimized extracorporeal circulation (MECC).

## Methods

Cardiac surgery and interventional cardiology procedures were performed according to institutional standards. Determination of inflammatory markers and CD62L shedding were performed after 4 (Study 2 and 3), 24 (Study 2 and 3) and 48 hrs (Studies 1, 2 and 3) post-operatively. The first blood sample (baseline value) was drawn before the procedures and prior to the application of anesthetic drugs.

The CD62L shedding assay. For the CD62L shedding assay 25 ml citrate whole blood was stimulated with 25 ml of six 10-fold dose-titrations starting at 10 mg/ml (Lipoteichoic acid, LTA), 20 ng/ml (tumor necrosis factor, TNF). After 45 min. incubation at 37 °C / 5 % CO<sub>2</sub>, cells were washed in PBS/BSA 1 % and stained with 25 ml PBS/BSA 1 % containing APC-anti-human CD33 and FITC-anti-human CD62L antibodies for 15 min. Red blood cells were lysed in 200 ml FACS Blood monocytes and granulocytes were distinguished on the basis of CD33 expression and side scatter. Median FITC fluorescence intensity is computed for each sample's granulocytes and monocytes and plotted against dilution

factor to enable parallel analysis of multiple agonists. Four-parameter curves are fitted using non-linear regression and LogEC50 values were extracted, corresponding to the dilution factor giving 50 % granulocyte or monocyte CD62L-shedding. The corresponding ligand concentration was calculated and plotted for each ligand.

*HLA-DR expression.* For human leukocyte (HLA-DR) expression 50  $\mu$ L of heparinized blood were stained with 20  $\mu$ L of anti-human-HLA-DR at room temperature in the dark for 25 min. Red blood cells were subsequently lysed with FACS lysis solution and washed twice with phosphate-buffered saline solution and fixed with 400  $\mu$ L of 4 % paraformaldehyde. The fluorescence intensity of the samples was measured on an LSR II as duplicates. A total of 500-1,000 monocyte events were recorded. The FACS data were analyzed with gating for CD14- and CD64-positive monocytes. The HLA-DR channel was calibrated using the data from the PE beads, which allows fluorescence intensity to be correlated with the mean number of PE molecules per cell. The results were recorded as the median of the calibrated PE channel fluorescence intensity of each sample.

Cytokine level measurement. Determination of cytokines (IL-6, IL-8, IL-10) was performed by ELISA in patients' plasma from each time point, which was separated from 5 mL of EDTA-whole blood by centrifugation at 3,000 g for 5 min and stored at -80 °C.

sCD62L level measurement. Soluble L-selectin (sCD62L) levels were measured by ELISA. The provided 96-well plate was treated according to the manufacturer's instructions, and all standards, controls and samples were loaded in duplicate. Optical density was measured using an eL800 microplate reader set to record at 450 and 630 nm. Blank values and OD values at 630 nm were subtracted from all OD 450 nm values, and the sCD62L-selectin concentration of all samples was determined by a 4-parameter curve fit of the standards.

sTLR-2 measurement. Soluble toll-like receptor (sTLR)-2 concentrations were detected using a human TLR-2 ELISA kit. The detection range (standard curve) was 0.312–20 ng/ml, intra- and inter-assay variation was given as 8 and 10 % CV by the manufacturer. For ADAM17 all samples were measured undiluted using a TACE human ELISA kit according to the manufacturer's

instructions. The detection range (standard curve) was 78.15 – 5000 pg/ml, intra- and inter-assay variation was given as 10 and 12 % CV.

## Results

Study 1. Assessment of the immune sensitivity in cardio-pulmonary bypass procedures using CD62L shedding assay. Granulocyte and monocyte sensitivity to LTA decreased at the end of cardiac surgery but re-covered to the baseline after 48 hrs. Decreased immune sensitivity was apparent as a roughly 10-fold increase of the initial LTA concentration was necessary to cause shedding of 50 % of CD62L from the cell surface of granulocytes or monocytes.

TNF stimulation of granulocytes showed a decreased sensitivity at the end of cardiac surgery as revealed by a 10-fold increased TNF concentration required to produce 50 % shedding of the membrane-bound fraction of CD62L. In contrast, monocyte sensitivity were not diminished at the end of surgery and after 48 hrs.

Median density of surface HLA-DR decreased significantly at the end of surgery. After 48 hrs monocyte density did not recover to the baseline, but rested reduced in comparison to the first sampling time point.

IL-8 levels significantly increased at the end of surgery as compared to the first sampling point. After 48 hrs. IL-8 decreased in comparison to the sampling point at the end of surgery, however, the preoperative value was not reached. The IL-8 concentration after 48 hrs was still significantly increased when compared to the first sampling time point.

sCD62L plasma levels significantly decreased at the end of surgery. After 48 hrs the levels were still lower as at the first sampling time point indicating that sCD62L did not reach pre-operative values after 48 hrs.

sTLR-2 showed a significant increase at the end of surgery. After this peak sTLR-2 levels decreased to a minimum at 48 hrs showing a significant difference to values at the end of surgery.

ADAM17 levels increased constantly during the peri-operative course, however, a significant difference between two sampling points cannot be detected.

Study 2. Investigation of inflammatory response to treatment of AS and Study 3. Investigation of inflammatory response to surgical treatment of AS.

The plasma level of HLA-DR showed a different course within the four treatment modalities. The highest baseline value of HLA-DR was detected in MECC patients. In this population HLA-DR decreased continuously having different values from the baseline at all sampling points. CECC, TF-TAVI and TA-TAVI patients exhibited much lower HLA-DR values. In CECC and TA-TAVI patients HLA-DR levels were different from the baseline at all sampling points. In TF-TAVI patients HLA-DR values decreased significantly from the baseline at 4 hrs. and 24 hrs. After 48 hrs, HLA-DR levels in TF-TAVI were not significantly different from the baseline.

The plasma level of IL-6 showed a different course within the four treatment modalities. The highest increase was detected in the TA-TAVI group peaking at 4 hrs post-operatively. TA-TAVI, MECC and CECC groups

peaked also within 24 hrs post-operatively, however at lower levels. The most attenuated IL-6 release was measured in the TF-TAVI group having only a slight increase in the observation period. In all groups IL-6 plasma levels were different from the baseline. At the end of the observation period no group returned to the pre-operative baseline. IL-8 showed a different course within the four treatment modalities. The highest increase was noted at 4 hrs post-operatively in the CECC group, followed by MECC group. TF-TAVI and TA-TAVI peaked at 4 hrs and 24 hrs. In the patient population undergoing interventional treatment, no significant differences were noted in the observation period. In contrast, the changes in the IL-8 plasma level in the CECC and MECC group were significantly different during the peri-operative course. In the MECC group IL-8 plasma level at 4 hrs and at 48 hrs was significantly different from the baseline. In the CECC group IL-8 was significantly different from the baseline at 4, 24 and 48 hrs. In contrast to the interventional treatment, in surgically treated patients IL-8 values did not return to the baseline at the end of the observation period. IL-10 did not show a different course within the four treatment

modalities. Highest IL-10 values were detected in the CECC group, followed by MECC treatment. In the interventional groups IL-10 peaked at 4 hrs with TA-TAVI and at 24 hrs with TF-TAVI, however with lower values as in the surgical groups. With interventional treatment IL-10 plasma levels were significantly different from the baseline at 4 hrs and at 24 hrs. In both groups IL-10 levels returned to the baseline at the end of the observation period. Likewise, IL-10 levels in MECC patients were not significantly different at the sampling points and at the end of the observation period. In the CECC population, IL-10 was different from the baseline at 4 hrs and at 24 hrs. At the sampling point at the end of the observation period, however, the IL-10 levels of CECC patients did not differ from the baseline.

sCD62L showed a different course within the four treatment modalities. Highest values were detected in the MECC, followed by the CECC group. In the MECC sCD62L values only at 4 hrs. and 48 hrs. were different from the baseline, whereas in the CECC sCD62L values at all sampling points were different from the baseline. Thus, surgically treated patients did not return with sCD62L to the baseline at the end of the observation

period. Interventionally treated patients did not show peaks in sCD62L levels in the observation period.

## Conclusions

- The results of this work indicate that SIRS and associated decreased immunity is present in all cardiac surgery and interventional cardiology procedures.
- The extent of peri-operative immunomodulation is detectable with both conventional inflammatory markers and the CD62L shedding assay. Moreover, the application of this assay provides additional information to the extent of peri-operative immunomodulation and contributes to a better understanding of peri-procedural inflammatory modulations.
- The impact of ECC on the inflammatory response is evident.
- Patients who are treated with CECC show the most pronounced inflammatory response.
- In patients undergoing TF-TAVI, only a diminished inflammatory response is detectable.

- The use of an extracorporeal circuit is not the sole factor that influences the extent of inflammatory molecule release.
- Likewise, patients pre-treatment condition and the extent of myocardial trauma affect the inflammatory course.
- The time-course and the extent of inflammatory response after a specific treatment modality do not predict the post-operative course, and there is no direct link to patient outcome.
- According to our results, the investigated variables (e.g., CECC, MECC, TAVI) represent only one factor amongst others that may influence the post-operative patient course.

### Publications related to PhD thesis

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