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Transient and permanent inflammation after implantation of non-degradable synthetic membranes and biological scaffolds

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Declaration

This doctoral thesis was carried out at the Department of Thoracic Surgery and the Department of Anaesthesiology, General Intensive Care and Pain Medicine, Division of Cardiac Thoracic and Vascular Anaesthesia and Intensive Care Medicine, Medical University of Vienna, in accordance with the guidelines of “Good Scientific Practice and the Declaration of Helsinki.” The Ethics Committee of the Medical University of Vienna approved these two studies. Written informed consent was obtained from all study participants including patients and volunteers.

I hereby declare that Dr. Cecilia Veraar exclusively wrote this doctoral thesis under supervision and guidance of Univ. Prof. Dr Hendrik Jan Ankersmit.

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Zusammenfassung

TEIL I – Perioperative Inflammation bei großen thoraxchirurigschen Eingriffen unter extracorporaler Zirkulation

Die elektive extrakorporale Membranoxygenierung (ECMO) ist heute ein fixer Bestandteil moderner Lungentransplantation. Die Anwendung von extrakorporalen Verfahren, wie der ECMO und dem kardiopulmonalen Bypass (CPB) während thorakokardialen Eingriffen, induziert jedoch eine Entzündungsreaktion. Die chemische und klinische Auswirkung dieser Reaktion auf die perioperative Mortalität und Morbidität dieser Patientinnen bleibt bisher jedoch unklar.

Ziel dieser Studie ist es, die perioperative Inflammation großer thoraxchirurgischer Eingriffe an extrakorporalen Verfahren anhand der Zytokinausschüttung zu messen und klinisch an einem etablierten Scoringsystem, dem sogenannten SOFA score, zu bewerten. Die Auswirkungen dieser perioperativen Entzündungsreaktion auf das 30 Tages Überleben und das auftreten postoperativer Komplikationen wird in weiterer Folge analysiert. Drei unterschiedliche thoraxchirurgische Operationen unter, und ohne Anwendung von extrakorporalen Verfahren werden beobachtet: Die Lungentransplantation an der ECMO, die pulmonale Endarterektomie am CPB und die Lungenresektionen ohne Einsatz einer extrakorporalen Zirkulation.

Es werden 42 konsekutive Patientinnen innerhalb eines Jahres von Mai 2018 bis April 2019 mit einer schweren Lungenerkrankung im Endstadium, die eine Lungentransplantation benötigen, eingeschlossen. Fünfzehn Patientinnen mit chronischer thromboembolischer pulmonaler Hypertonie, die sich einer pulmonalen Endarteriektomie unterziehen, und 15 Patienten mit Lungentumor, die sich einer großen Lungenresektion benötigen. Zytokin-Serumkonzentrationen und SOFA werden vor, am Ende der Operation und in den darauf folgenden 5 postoperativen Tagen bestimmt.

Die Lungentransplantation an der ECMO und die pulmonale Endarteriektomie unter Anwendung eines CPB lösen postoperativ, im Vergleich zum Ausgangswert, präoperativ, einen sofortigen Anstieg der Zytokin-Serumkonzentrationen aus: IL-6: 66-fach und 71-fach, IL-10: 3-fach und 2,5-fach , ST2/IL-33R: 5-fach und 4-fach und SOFA: 10,5 ± 2,8 und 10,7 ± 1,7. Dieser Anstieg reduziert sich stetig in den folgenden 5 postoperativen Tagen.

Obwohl in dieser Pilotstudie eine sehr niedrige perioperative Mortalität (3 Patienten, 4,1%) zu beobachten ist, zeigt sich eine Korrelation zwischen extrem hohem SOFA≥13 und einer erhöhten perioperativen Mortalität nach Lungentransplantation.

Die postoperative relative Abnahme im SOFA score, genannt Delta-SOFA, hilft uns Überlebende von Nicht-Überlebenden zu unterscheiden. der Abfall im Delta-Sofa ist bei Überlebenden signifikant steiler: -4,5 ± 3,2 gegenüber -0,3 ± 1,5 ($p = 0,001$). Erhöhte IL-6-Serumkonzentrationen sind prädiktiv für erhöhten SOFA (Sensitivität: 97 %, Spezifität: 80 %). Die maximalen Zytokin-Serumkonzentrationen korrelierten mit der Dauer des extrakorporalen Ersatzverfahrens, mit dem maximalen Laktatwert, Anzahl von verabreichten Erythrozyten Konzentraten, sowie frisch-gefrorenem Plasma und der Katecholamin-Unterstützung.

Lungentransplantation und pulmonale Endarteriektomie am extrakorporalen Kreislauf führen bei niedriger Mortalität und Morbidität, perioperativ zu einer akuten Entzündungsreaktion, die in den folgenden Tagen wieder abnimmt, wie laborchemisch an Zytokin-Serumkonzentrationen bestimmt wird und klinisch an einem ansteigenden und wiederabfallendem SOFA beobachtet werden kann. Es wird daher davon ausgegangen, dass eine perioperative klinische und laborchmisch bestimmte Entzündungsreaktion bei großen thoraxchirurgischen Operationen Merkmal eines komplexen chirurgischen und anästhesiologischen therapeutischen Vorgehens ist und kein Anzeichen eines Organ- beziehungsweise Therapieversagens ist.

TEIL II - Alpha-Gal spezifische und unspezifische Immunantwort auf Transkatheter-Aortenklappenimplantation

Seit der ersten kathetergeführten Implantation einer biologischen Herzklappenprothese im Jahr 2002 durch den französischen Kardiologen Alain Cribier hat sich die Transkatheter-Aortenklappenimplantation, abgekürzt TAVI stark weiter entwickelt. Heute ist die minimalinvasive TAVI ein therapeutischer Standard in der Behandlung von älteren PatientInnen mit hochgradiger, symptomatischer Aortenklappenstenose.

Bisher blieb es jedoch unerforscht, ob bioprothetische Herzklappen für TAVI ähnlich wie die chirurgischen Herzklappenprothesen das immunologisch aktive Galactose-Alpha-1,3-Galactose (alpha-Gal) Epitop tragen und in Folge eine Immunrekationen auslösen.

Daher untersuchten wir in dieser prospektiven Kohortenstudie, ob bioprothetische Herzklappen für TAVI eine alpha-Gal-spezifische Antikörper-abhängige und eine Antikörper-unabhängige Immunantwort 3 Monate nach der TAVI-Implantation auslösen.

In dieser prospektiven Beobachtungsstudie wurden zwischen März und August 2019 27 PatientInnen mit schwerer Aortenklappenstenose, die sich einer TAVI unterziehen und 10 PatientInnen mit schwerer Mitralklappeninsuffizienz, die mit einem Transkatheter-MitraClip-Verfahren behandelt wurden, analysiert. Blutproben wurden vor und 90 Tage nach der Behandlung bei einer Routineuntersuchung abgenommen. Serumproben wurden unter Verwendung des enzymgebundenen Immunosorbent-Assays, abgekürzt ELISA, analysiert. Die Serumkonzentrationen von alpha-spezifischem Immunglobulin (Ig) G, IgG-Subklassen und IgE, Komplementfaktor C3a, NETose-spezifischem citrulliniertem H3 (citH3), ST2 und IL-33 wurden gemessen und statistisch ausgewertet.

Drei Monate nach TAVI fanden wir signifikant erhöhte Serumkonzentrationen von alpha-Gal-spezifischem IgG3, Komplementfaktor C3a, citH3-Spiegel und ST2 ($p=0.002$, $p= 0.001$, $p=0.025$ und $p=0.039$). Eine Sensibilisierung von alpha-Gal-spezifischen IgE-Antikörpern trat bei 55 % aller Patienten nach TAVI auf.

Unsere Ergebnisse zeigen, dass eine TAVI eine mittelfristige, spezifische humorale Immunantwort gegen alpha-Gal auslöst und im Vergleich zu Patientinnen nach MitraClip-Implantation eine unspezifische humorale Entzündung verursacht. Diese Beobachtung hilft uns weiter im Verständnis postinterventioneller Morbidität und weist uns auf Unsicherheiten in der langfristigen Haltbarkeit von Bioprothesen hin. Für Implantationsstrategien von bioprothetischen Herzklappen für TAVI muss Vorsicht in jüngeren Patientinnen geboten sein.

Summary

PART I - Transient perioperative inflammation during major thoracic surgery on extracorporeal circulation

The impact of inflammation on clinical short- and long-term outcome during extensive thoracic surgery on Extracorporeal Circulation has to be further evaluated.

This study aimed to investigate the qualitative and quantitative perioperative cytokine “storm” during different surgical procedures with or without extracorporeal support.

We therefore analyzed 42 consecutive patients with end-stage respiratory disease such as chronic obstructive lung disease, idiopathic pulmonary fibrosis, idiopathic pulmonary hypertension and cystic fibrosis undergoing lung transplantation; fifteen patients with chronic thromboembolic pulmonary hypertension undergoing pulmonary endarterectomy and 15 patients with lung cancer undergoing lobectomy. Cytokine serum concentrations and SOFA were measured before, at end of surgery and during the consecutive postoperative 5 days.

Lung transplantation on extracorporeal membrane oxygenation and pulmonary endarterectomy on cardiopulmonary bypass induced both an immediate increase of cytokines directly after surgery: IL-6 increased 66-fold and 71-fold, IL-10 rose 3-fold and 2.5-fold, ST2/IL-33R increased 5-fold and 4-fold and SOFA went up to 10.5 ± 2.8 and 10.7 ± 1.7. During the following postoperative day 1-5 cytokines and SOFA decreased back to baseline levels. Extremely high SOFA≥13 was associated with adverse short-term outcome after lung transplantation. Delta-SOFA, calculated as a relative change from end of surgery to postoperative day 3 was significantly decreased in survivors compared to non-survivors: -4.5±3.2 versus -0.3±1.5. Elevated IL-6 levels were predictive for high SOFA, with a sensitivity of 97% and a specificity of 80%.

High peak cytokine concentrations were significantly associated with increased ECC time, high maximal lactate levels, an increased catecholamine support and a high rate of packed red blood cell and fresh frozen plasma transfusion.

Lung transplantation and pulmonary endarterectomy on extracorporeal circulation trigger an immediate increase and concomitant fall of inflammation as determined in cytokine concentrations and SOFA. The perioperative inflammatory “storm” induced by major thoracic surgery seems to reflect management strategies rather than pointing out adverse events.

PART II - Alpha-Gal specific Immune Response after TAVR

Transcatheter aortic valve replacement (TAVR) has become a safe and minimal invasive therapeutic choice for older patients with severe aortic valve stenosis who are at high operative risk or unsuitable for surgery. However, investigations on mid- and long-term biocompatibility of alpha-Gal carrying bioprosthetic heart valves for TAVR remain outstanding. We therefore aimed to analyze whether bioprosthetic heart valves employed for TAVR enhance an alpha-Gal specific and unspecific immune response 3 months after the TAVR procedure. We studied 27 consecutive patients with severe aortic valve stenosis treated with TAVR and 10 patients with severe mitral valve regurgitation undergoing transcatheter MitraClip procedure, serving as a reference group. Alpha-Gal specific immunoglobulin (Ig)G, IgG subclasses and IgE, complement factor 3a (C3a), NETosis-specific citrullinated H3 (citH3) and the systemic inflammation marker soluble suppression of tumorigenicity (sST2) were measured before and 90 days after the TAVR or MitraClip procedure. Three months after TAVR, alpha-Gal–specific IgG3, C3a, citH3 and sST2 were significantly elevated. Alpha-Gal–specific IgE antibodies occurred in 55% of all included patients after TAVR procedure.

Our findings suggest that TAVR evoke a midterm, alpha-Gal specific and unspecific humoral immune reaction compared to patients undergoing a MitraClip procedure. This observation is important to understand post-interventional morbidity after TAVR and should attract attention when creating implantation strategies for younger patients.

Publications arising from this thesis

Manuscript 1

Title: Transient perioperative inflammation following lung transplantation and major thoracic surgery with elective extracorporeal support: a prospective observational study

Authors:

Cecilia Veraar, Stefan Schwarz, Jürgen Thanner, Martin Direder, Panja M. Boehm, Leopold Harnoncourt, Joachim Ortmayr, Clarence Veraar, Julia Mascherbauer, Walter Klepetko, Martin Dworschak, Hendrik J. Ankersmit, Bernhard Moser

Contribution by the doctoral applicant:

Study design, conduct of study, data analysis, manuscript preparation, manuscript revision

Reference in PubMed:

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Manuscript 2

Title: Inflammatory immune response in recipients of transcatheter aortic valves

Authors:

Cecilia Veraar, Matthias Koschutnik, Christian Nitsche, Maria Laggner, Dominika Polak, Barbara Bohle, Andreas Mangold, Bernhard Moser, Julia Mascherbauer and Hendrik J. Ankersmit,

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List of abbreviations

PART I

ACR	Acute cellular rejection
AMR	Antibody mediated rejection
ARAD	Azithromycin-Responsive-Allograft-Dysfunction
ARDS	Acute respiratory distress syndrome
ATG	Antithymocyte globulin
AZA	Azathioprine
BAS	Balloon atrial septostomy
BO	Bronchiolitis Obliterans
BOS	Bronchiolitis obliterans syndrome
BPA	Balloon pulmonary angioplasty
CABG	coronary artery bypass graft
CF	Cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CO	Cardiac output
CO_2	Carbon dioxide
CPB	Cardiopulmonary bypass
CT	Computed tomography
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CCB	Calcium channel blocker
CLAD	Chronic lung allograft dysfunction
COPD	Chronic Obstructive Pulmonary Disease
CTEPH	Chronic Thromboembolic Pulmonary Hypertension
CPB	Cardiopulmonary Bypass
Δ-SOFA	Relative change of SOFA, delta SOFA
DHCA	Deep hypothermic circulatory arrest
DLUTX	Double lung transplantation
dnDSA	de novo
DSA	Donor specific antibody
ECC	Extracorporeal Circulation

$ECCO_2R$	Extracorporeal CO_2 removal
ECM	Extracellular matrix
ECMO	Extracorporeal Membrane Oxygenation
ECLS	Extracorporeal life support
ELISA	Enzyme-linked immunosorbent assay
ERA	Endothelin-receptor antagonist
ERS	European Respiratory Society
ESRD	End-stage renal disease
ESC	European Society of Cardiology
ET-1	Endothelin-1
FEV1	Forced Expiratory Volume in one second
FVC	Forced vital capacity
FFP	Fresh Frozen Plasma
gp	Glycoprotein
GC	Guanylate cyclase stimulator
HIV	Human immunodeficiency virus
HIT	Heparin-induced thrombocytopenia
HAR	Hyperacute transplant rejection
HFpEF	Heart failure with preserved ejection fraction
HFrEF	Heart failure with reduced ejection fraction
HLA	Human leukocyte antigen
HSP	Heat shock protein
HTX	Heart transplantation
ICU	Intensive care unit
IL	Interleukin
IL2RA	Interleukin-2 receptor antagonists
IRT	Immunoreactive trypsinogen
ICS	Inhaled corticosteroids
iNO	Inhaled nitric oxide
IPAH	Idiopathic Pulmonary Arterial Hypertension
IPF	Idiopathic Pulmonary Fibrosis
LAMA	Long-acting antimuscarinic antagnosist
LABA	Long-acting Beta2-agonist

LAS	Lung Allocation Score
LUTX	Lung Transplantation
LVAD	Left ventricular assist device
LVRS	Lung volume reduction surgery
metHb	Methaemoglobin
MMF	Mycophenolate mofetil
MRI	Magnetic resonance imaging
MODS	Multiple organ dysfunction syndrome
NPV	Negative predictive value
NO	Nitric oxide
OD	Optical density
PDE	Phosphodiesterase
PEA	Pulmonary Endarterectomy
PGD	Primary Graft Dysfunction
PGI2	Prostacyclin
PPV	Positive predictive value
PVR	Pulmonary vasculature resistance
qSOFA	quickSOFA
RAS	Restrictive Allograft Syndrome
SABA	Short-acting Beta2-agonist
SAMA	Short -acting antimuscarinic antagnosist
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential Organ Failure Assessment
sST2	Soluble ST2
ST2L	ST2 ligand
TACAD	Transplant-associated coronary artery disease
TIVA	Total intravenous anesthesia
TEE	Transesophageal echocardiography
TGF	Transforming Growth Factor
TNF	Tumour Necrosis Factor
TMB	Tetramethylbenzidine
UIP	Usual interstitial pneumonia
va	Veno-arterial

vv Veno-venous

PART II

Alpha-Gal	Galα1.3–Galβ1–4GlcNAc–R
AB	antibody
ACC	American College of Cardiology
ACL	anterior cruciate *ligament*
ACR	acute cellular rejection
ACEI	Angiotensin-converting enzyme inhibitors
AHA	American Heart Association
AHXR	acute humoral xenograft rejection
ARB	angiotensin II receptor blockers
AS	aortic stenosis
BHV	bioprosthetic heart valves
BTB	bone-patellar-tendon-bone
citH3	citrullinated histone H3
CAD	coronary artery disease
DAPI	40 ,6-diamidino-2-phenylindole
EF	ejection fraction
ELISA	enzyme-linked immunosorbent assay
EuroSCORE	European System for Cardiac Operative Risk Evaluation
HAR	hyperacute xenograft rejection
LPS	lipopolysaccharide
NE	neutrophil elastase
Neu5Gc	*N*- glycolylneuraminic acid
NK cells	natural killer cells
NYHA	New York heart association
mAB	monoclonal antibody
MR	Mitral regurgitation
SAVR	Surgical aortic valve replacement
STS-PROM	Society of Thoracic Surgeons- predicted risk of mortality
TAVR	Transcatheter aortic valve replacement
TEER	transcatheter edge-to-edge repair
pEC	porcine endothelial cells
PMA	phorbol 12-myristate 13-acetate
WHO	world health organisation

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Prologue

Synthetic and biological materials in medical practice

Biomaterials became a key component of modern clinical practice and form the basis of several permanent or short-term treatments comprising pacemakers, artificial articulating joints, catheters, sutures, biological and mechanical heart valves, transient usage of extracorporeal membrane oxygenation (ECMO)s, left ventricular assist devices (LVAD)s and many others.[1] All these biomaterials that are implanted within the body will induce an immune response. This process diverges among materials according to their surface chemistry, stiffness and degradability.[2] After some implantations such as pacemakers or artificial articulating joints the desired immune reaction would be ignorance or tolerance. In contrast, in regenerative medicine an immune response is desired to help integrating the scaffold with the surrounding tissue and grow new tissue.[3]

Biomaterials can be broadly classified as synthetic and biological derived substances.[1] Synthetic materials can be further divided in non-degradable and degradable and are primarily composed out of chemically manufactured polymers such as polyesters, polyethers and ceramics, with the advantage that implants can be easily reproduced and manufactured with a wide range of chemical and physiological properties.[4] In contrast, biological materials are composed out of biopolymers such as proteins and polysaccharides.[5, 6] These naturally occurring allogeneic or xenogeneic scaffolds composed of extracellular matrix (ECM) are prepared by removing all cellular contents from the source tissues while preserving the structural and functional molecular units of the ECM.[7]

Immune response to synthetic materials

The immunological response to synthetic materials was studied for the first time in the 1980s to comprehend the formation of fibrous capsules around implants known as the classical foreign body response (FBR), which begins with protein adsorption and neutrophil migration to the newly implanted materials followed by inflammatory macrophage recruitment and secretion of oxide radicals, formation of foreign body giant cells and ultimately fibrosis.[1, 8] Anderson and colleagues discovered more details on the FBR by implanting stainless steel wire mesh cages with a polymer specimen subcutaneous into the back of rats. After implantation, primary neutrophils were migrating, followed by infiltrating monocytes/macrophages and finally fibroblasts mediate the process of remodeling via collagen deposition and capillary bed formation. This cage implant

system allowed the isolation of various cell types that interacted with the biomaterials and helped to define the basic process of the FBR.[9]

Immune response to biological scaffolds

In contrast to synthetic materials, biological scaffolds do not induce an FBR response.[10] Until now, the immune reaction to biological scaffolds remains only partially understood.[10] Since, almost all xenogeneic antigens are cell-associated, decellularization has to be performed to remove all cells and cell remnants of the ECM. Without decellularization cells from an allogeneic or xenogeneic scaffold would be recognized as foreign and initiate both innate and acquired immune responses resulting in inflammation at a minimum, and more commonly, outright rejection. Therefore, the efficacy of decellularization is a major determinant of the host response post implantation of ECM scaffolds.[11] Due to remarkable homology of amino acid sequences of the ECM across species, the immune response in recipients of decellularized scaffolds was reported as low.[12] However, there are two very well known antigens within the ECM of almost all species except of humans and primates: the Alpha-Gal epitope and the N-glycolylneuraminic acid (Neu5Gc).[13-15]

Both, the innate and adaptive arms of the immune system cross-talk in the process of remodeling of ECM scaffolds: The host cellular response consists of an immediate infiltration of neutrophils with a small number of macrophages, followed by a rapid transition to an almost exclusive mononuclear cell infiltrate by 4–7 days post-implantation.[12, 16] Macrophages are now recognized as cells with a remarkable plasticity and play a major role in orchestrating pro-inflammatory responses and constructive wound-healing on the other hand.[17, 18] Functional subgroups of macrophages are now discovered comprising a pro-inflammatory M1-like and an anti-inflammatory M2-like phenotype with homeostatic, wound-healing and regulatory properties.[19-22]

The polarization of macrophages toward particular effector functions is regulated by microenvironmental cues.[23] The extent to which biological scaffolds influence the polarization of these macrophages determine healing outcomes. A positive correlation between the local tissue ratio of M2-like macrophages to M1-like macrophages (M2:M1) has been shown to be associate with constructive, functional outcomes for a large number of commercially available biological scaffold mesh devices.[24] Normal wound healing in most tissues starts with predominantly pro-inflammatory M1-like macrophages, which then transit into a more prominent M2-like profile.[12] The transition toward M2-like macrophages suppresses pro-inflammation and supports constructive wound healing. An absent

transition of M1-like to M2-like macrophages results in persistent and chronic inflammation.[25] Together with synthetic materials, biological scaffolds can be divided into degradable and non-degradable materials. Macrophages are mainly important for in vivo degradation and remodeling of biological scaffolds.[26]

Preclinical studies have shown a strong positive correlation between ECM scaffold degradation, an increased M2:M1 ratio, and constructive remodeling outcomes.[27, 28] To develop non-degradable ECM scaffolds chemical crosslinking agents are necessary to alter the native structure and composition of the matrix. However, modification inhibits the beneficial M2, thereby inducing a chronic pro-inflammatory condition.[28] The humoral immune response is known as a further barrier for short- and long-term use of xenogeneic biological scaffolds.[29]

Following implantation of xenogeneic ECM pre-existing or de novo antibodies trigger their deposition, complement activation, generation of chemotactic complement fragments and recruitment of polymorphonuclear leukocytes and macrophages.[29] During degeneration of damaged bioprosthetic heart valves both macrophages and neutrophils were activated through IgM, IgG and iC3b.[29-31] After islet xenograft transplantation an antibody-dependent activation of the classical complement cascade, has been found to contribute to early degradation and rejection.[29, 32] Both, carbohydrates linked to proteins and lipids, and protein epitopes are known targets of the humoral anti-xenograft response. Especially, alpha-Gal, but also non-alpha Gal residues are known targets of the natural and elicited humoral immune response as will be discussed in detail in this thesis.[33]

Purpose of the present doctoral thesis

In this doctoral thesis we aim to investigate 1) the impact of transiently introduced synthetic non-degradable materials during major thoracic surgery comprising ECMO and coronary-bypass graft (CPB) insertion during lung transplantation and pulmonary endarterectomy; and 2) the consequence of permanently implanted biological non-degradable scaffolds such as biological heart valves on the immune systems of patients with severe aortic valve stenosis receiving transcatheter aortic valve replacements.

PART I – Transient perioperative inflammation during major thoracic surgery on extracorporeal circulation

CHAPTER ONE: INTRODUCTION

1.1 Inflammation

1.1.1 Concept of SIRS and CARS

In 1991 and 1992, the American College of Chest Physicians and the Society of Critical Care Medicine defined systemic inflammation that was recognized to accompany severe infection and sterile trauma, and introduced the term systemic inflammatory response syndrome (SIRS).[34] At that time, the theory that solely SIRS accompanies severe infection and sepsis by an uncontrolled hyper-inflammatory response was prevailing.[35] In 1996, Roger Bone, who had been the chair of the Consensus Conference back in 1991, introduced a second acronym to portray the host response during sepsis, the compensatory anti-inflammatory response syndrome (CARS); to qualify the consequence of counter-regulatory mechanisms initiated to limit the overzealous inflammatory process in patients with infectious (septic) or non-infectious SIRS.[36] Bone hypothesized that either SIRS or CARS could predominate in a given patient and both events occur concomitant.[36] In contrast, other authors postulated that CARS follows SIRS in a two-wave process.[37] Recently, Hotchkiss and colleagues confirmed Bone's hypothesis that activation of both pro- and anti-inflammatory immune responses occur simultaneously and supported the concept of even pronounced sepsis-induced immunosuppression with T cell exhaustion and numerous other defects in host innate and adaptive immunity. Thereby suggesting that "sterile" and "non-sterile" septic patients become even more susceptible to intractable infection or new secondary infections.[38]

1.1.2 Cytokines

Cytokines orchestrate these inflammatory immune responses, comprising SIRS and CARS to combat tissue injury and infection, aiming to restore normal conditions and tissue architecture.[39, 40] Thereby, the so-called “immunological switch”: the transition from innate to acquired immunity remains essential.[39, 40]

Interleukin-6

IL-6 plays a cardinal role in these processes by controlling leukocyte recruitment, activation, and apoptosis. Further IL-6 is considered as highly relevant in acute phase reactions and by stimulating lymphocyte stimulatory factors. [40] IL-6, first described as a soluble factor in 1973 by Kishimoto and colleagues, belongs to a cytokine family that promotes cellular processes. IL-6 can bind to either a membrane-bound cognate receptor (IL-6R) or to a soluble (s) IL-6R and forms receptor complexes with the signal-transducing glycoprotein (gp) 130.[41, 42] The classical pathway, binding of IL-6 to IL-6R is limited to cells with a membrane-bound receptor, since IL-6R is solely found on hepatocytes and several leukocyte subpopulations.[43] The IL-6R and its ligand IL-6 form an IL-6•IL-6R complex, which then associates with membrane- bound gp130. Gp130 is expressed ubiquitously on every cell type. *The classic-signaling* process is linked to regeneration and anti-inflammatory conditions.[44] sIL-6R binds free IL-6 and forms IL-6•sIL-6R complexes, which again bind to membrane-bound gp130 and cause IL-6 trans-signaling. *The trans-signaling* process mediates pro-inflammatory effects of IL-6 such as higher endothelial permeability.[45] Similar to sIL-6R, a soluble form of gp130 (sgp130) is circulating in plasma. Sgp130 can bind and neutralize the IL-6•sIL-6R complex and subsequently inhibits pro-inflammatory conditions.[46] This natural occurring IL-6 buffer is dependent on sIL-6R serum concentrations and is able to antagonize low concentrations of circulating IL-6.[47]In physiological conditions, IL-6, sIL-6R, and sgp130 are in equilibrium.[48]

TNF-alpha

In 1975 TNF-alpha was mentioned for the first time; it was descripted as an endotoxin-induced glycoprotein causing haemorrhagic necrosis in sarcomas, transplanted into mice.[49] Ten years thereafter human TNF-alpha was cloned for the first time.[50] Since then, TNF-alpha has been found in various infectious, inflammatory and malignant diseases. The significance of TNF-alpha in inflammatory conditions has been emphasized by the

efficiency of anti-TNF antibodies and soluble TNF receptors (TNFRs) in controlling disease progression.[51] Macrophages and T lymphocytes produce pro-TNF-alpha, which remains on the plasma membrane of these cells. Subsequently, TNF-alpha can be cleaved into an extracellular domain, known as soluble.

Membrane-associated and soluble TNF-alpha have different biological activities. However both conformations are solely active in their trimeric form.[52] In healthy individuals TNF-alpha is usually not detectable. In contrast, in patients with inflammatory and infectious diseases increased serum and tissue concentrations were measured.[53] Furthermore, serum concentrations of TNF-alpha correlate with disease severity.[54] Even though, TNF-alpha is mainly produced by macrophages, several other cell types can produce TNF-alpha, comprising T and B lymphocytes, NK cells, mast cells, neutrophils, endothelial cells, smooth and cardiac muscle cells, osteoclasts and fibroblasts.[51]

The regulation of TNF-alpha signal transduction is a very complex process, which remains barely understood. NF-κB is known as the cornerstone of TNF-alpha signal transduction. All reported responses to TNF-alpha were triggered via binding of TNF-alpha to either TNFR1 or TNFR2 as depicted in **Figure 1**. Pro-inflammatory and programmed cell death pathways were activated via TNFR1. Contrary, activation of TNFR2 promotes angiogenesis and tissue repair. [55]

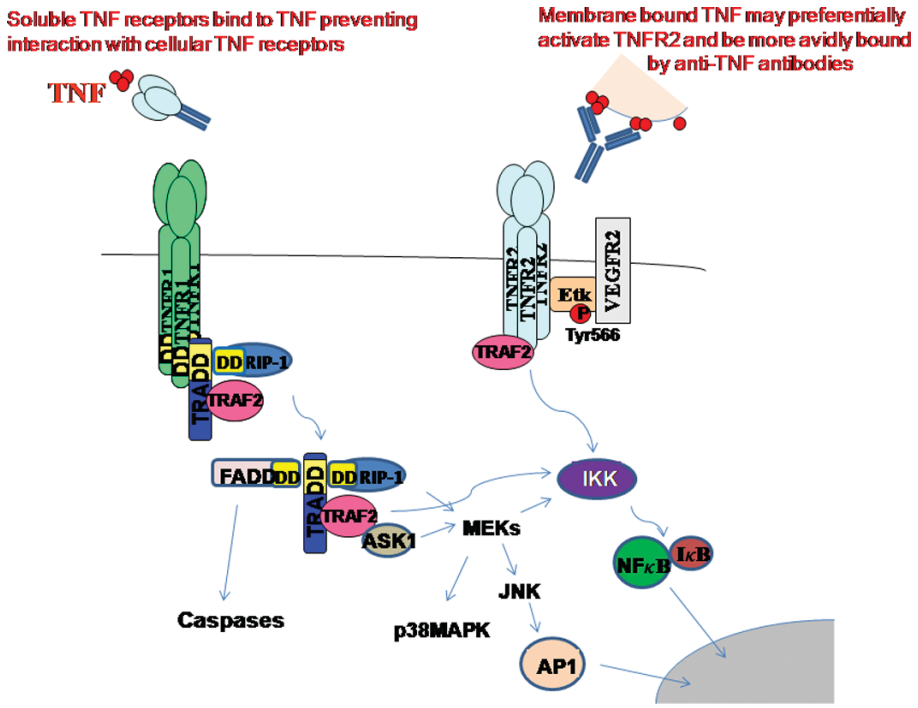

Figure 1: Signalling pathways of TNF-alpha.

Soluble TNF receptors or monoclonal anti-TNF antibodies prevent TNF interacting with its receptors and activating these pathways. Adapted from Bradley and colleagues.[51]

Looking closer to the vascular endothelium and to endothelial leukocyte interactions can elucidate pro- inflammatory actions of TNF-alpha. TNF-induces COX 2 production and increases PGI2 expression resulting in vasodilatation, leading to rubor and calor due to increased local blood perfusion.[51, 56] Even though several studies found a link between increased TNF-alpha and disease pathogenesis, clinical trials failed to demonstrated a clinical benefit of TNF-alpha blockade.[57]

IL-10

IL-10 is a pleiotropic cytokine that has traditionally been considered to exert immunosuppressive effects by inhibiting the production of pro-inflammatory cytokines.[58] The inhibitory functions of IL-10 explain its original name: "cytokine synthesis inhibitory factor."[59] Even though Th2 cells were the first identified IL-10 producing cells.[59] the production of this cytokine by other CD4+ and also CD8+ T cells was soon demonstrated.[60] Today we know that cells of both the myeloid and lymphoid lineages secrete IL-10 in response to different stimuli, including macrophages, monocytes, dendritic cells, neutrophils, mast cells, eosinophils, and natural killer cells, in addition to CD4+, CD8+ T cells and B cells.[61] More recently it was found that even tumor cells produce IL-10 to induce immunosuppression.[62] Besides, IL-10 can be activated via systemic release of TNF-alpha, a NF-κB-dependent pathway.[63] IL-10 can be activated via two different receptors: IL-10R1 and IL-10R2. The first mentioned receptor, IL-10R1 has a high affinity to IL-10, whereas IL-10R2 has a low affinity. Therefore, IL-10 first binds to IL-10R1 and subsequently to IL-10R2.[64] IL-10 has two different roles: on one hand IL-10 inhibits cell- mediated immunity and on the other hand IL-10 enhances humoral immunity.[65]

As a TH2 cytokine, IL-10 antagonizes TH1 lymphocytes by suppressing the TH1 cytokine expression including TNF-alpha, IL-6, IFN-γ, IL-12 and IL-18 as depicted in **Figure 2**.[66] IL-10 seems to neutralize inflammation by reducing duration and magnitude of the ongoing inflammatory process. An animal study on mice showed that IL-10 knockout mice and normal mice treated with IL-10 antibodies had an exaggerated inflammatory response to induced endotoximea or peritonitis.[67] Additionally, in the same study they found that IL-10 knockout mice had an overproduction of inflammatory cytokines and more often developed chronic inflammatory diseaseses.[68] Interestingly, these inflammatory cytokines were manly transcriptionally controlled by the transcription factor

NF-κB. Accordingly the study group concluded that IL-10 exerts its anti-inflammatory response by inhibiting NF-κB. [69]

Further anti-inflammatory reactions of IL-10 are to inhibit the release of ROS and to down-regulate NO prodcution.[70,71]Corticosteroids, are known to exert their immunosuppressive properties by increasing the plasma concentration of IL-10.[72]

In 1990 the mouse and human IL-10–coding genes were cloned, thereby researches discovered that the protein sequences of IL-10 and the Epstein-Barr virus were highly homologous.[74] This was the first discovery of viruses exploiting the inhibitory properties of IL-10 as a mechanism of immune evasion.[75] Soon thereafter, several other viruses were found to encode a homologue of the IL10 gene.[76] Several studies examined IL-10 concentrations in the blood of patients with sepsis; and demonstrated that blood IL-10 concentrations were extremely labile, with levels varying between 12 and 2400 pg/mL in patients with sepsis. Besides, patients with septic shock show significantly higher IL-10 concentrations compared to septic patients.[77] In patients with meningococcal sepsis, blood IL-10 levels even exceeded 20,000 pg/mL.[78] Further, correlations were found among TNF-alpha and IL-10 serum concentrations and Friedman and colleagues could even report that plasma IL-10 concentrations are able to predict the incidence and severity of MODS.[79, 80] Experimental works showed that TNF-alpha administration to healthy adults induced a monophasic IL-10 response, peaking 45 minutes thereafter.[81]

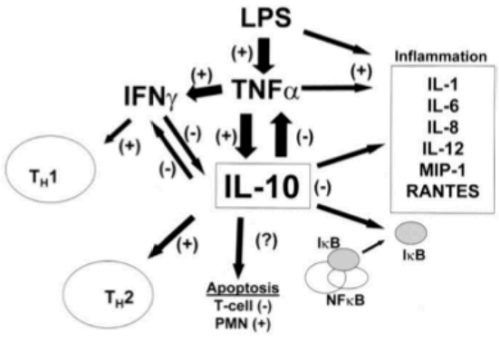

Figure 2: Regulation of IL-10 synthesis by TNF-alpha and endotoxin.

TNF-α and endotoxins are potent inducers of IL-10 synthesis. Nevertheless, IL-10 down-regulates TNF-alpha and other pro-inflammatory cytokines. Adopted from Oberholzer and colleagues. [66]

LPS lipopolysaccharide; *IFN* interferon; *NF* nuclear factor; *PMN* polymorphonuclear, *RANTES* regulated upon activation—normal T cell expressed and secreted.

ST2

ST2 is a protein of the interleukin-1 receptor family. Alternative splicing of the ST2 gene generates three mRNAs, corresponding to a longer membrane- anchored form (ST2L), a shorter released form (soluble ST2), and a membrane bound variant form (ST2V).[82] Activation of the membrane-bound ST2-receptor via IL-33 activates NFκB and ultimately, the expression of T-helper cell (Th2) associated cytokines comprising IL-4, IL-5 and IL-13 and enhances the pro-inflammatory cytokine production of IL-8 and IL-6.[83] Therefore a potential role for IL-33 may be to alert the immune system of cell or tissue damage, as an endogenous 'danger' signal or a so called 'alarmin' after the release from dead or dying cells during trauma or infection as described by Moussion.[84] sST2 acts as a so-called *decoy receptor* for IL-33 by capturing IL-33 to reduce Th2 inflammatory responses.[86] sST2 interacts with macrophages and suppresses pro-inflammatory proteins by down regulating NFκB. [87, 88] Mildner et al. found that human myocytes and pneumoepithelial cells are the main sources of circulatory bioactive sST2 and that endotoxin triggering, via induction of inflammatory cytokines in PBMC, augment sST2 secretion in alveolar epithelial cells and cardiac myocytes.[85] The authors assumed that lung cells may require sST2 to ensure endotoxin tolerance, since the lungs have an enormous surface, which is exposed to endotoxins.[88] Furthermore, it has been reported massively enhanced sST2 secretion after heart surgery.[87] Besides, sST2 was identified as a biomarker for cardiac heart failure.[89] Recently, in 2021 Lingitz and colleagues investigated the impact of an infection with Coronavirus Disease 2019 on sST2 secretion. The study group found that immune suppressive sST2 cytokine serum concentrations were massively enhanced in patients with COVID-19. Further, sST2 levels were associated with mortality, invasive ventilation, and oxygen support. The authors concluded that Coronavirus Disease 2019 may be more likely explained by immunodeficiency than by hyper-inflammation.[90] Another study investigated circulating IL-33 and sST2 in each trimester of normal pregnancy and in women with pre-eclampsia. While IL-33 did not change throughout normal pregnancy, or between non-pregnant, normal pregnant or pre-eclamptic women, sST2 was significantly increased in the third trimester of normal pregnancy and was further increased in pre-eclampsia. This increase in sST2 cytokine serum concentrations was even seen before disease manifestation.[91]

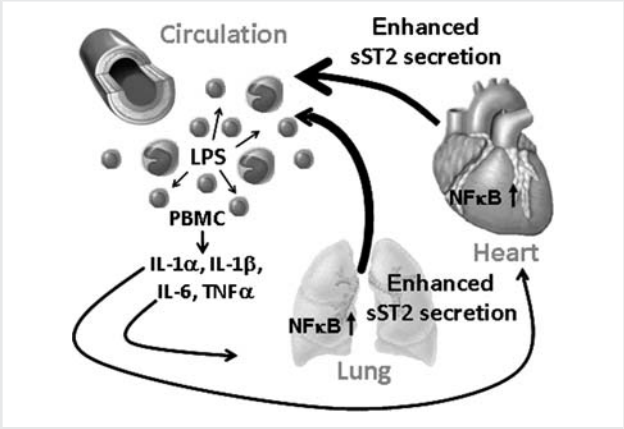

Figure 3: Scheme of the human endotoxin model.

Endotoxin stimulation induces inflammatory mediator production of IL-1a, IL-1b, IL-6, and TNF-alpha. Inflammatory cytokines stimulate sST2 via NFkB in lung alveolar epithelial cells and cardiac myocytes. Secreted sST2 enters the circulation and consequently attenuates immune responses in organs exposed to external and internal antigenic triggers. Adopted from Mildner and colleagues.[85]

1.1.3 Previous works of our study group

Inflammation and sepsis

Increased T-cell apoptosis as descripted above is also known as an immunological response to sepsis.[92] In line with Moser and colleagues who found as already mentioned above that there is a predominance of Th2 derived cytokines in chronic renal failure, several researches found that there is also a predominate Th2 antibody-mediated immune responses in sepsis patients.[93, 94] Besides, several autoimmune disease have a similar Th2 cell-type immune response.[95] At that time, ST2 was a novel Th2 specific protein.[96] Since, ST2 genes were only expressed on activated Th2 cells, but not on Th1 cells, they play a pivotal role in Th2 effector functions, such as elevated cytokine levels of IL-4, IL-5, and IL-13.[97]

Since sepsis was shown to increase the production of anti-inflammatory Th2 cytokine, Brunner and colleagues investigated the presence of sST2, Th1-Th2 cytokines, and the Ig content of critically ill patients.[93] Therefore, they included 15 patients within 24–48 h after diagnosis of sepsis, 13 trauma patients within 24 h after admission to the ICU, 11 patients who underwent abdominal surgery, and 15 healthy volunteers who served as a control group. [98] In contrast to trauma, abdominal surgery and healthy controls, septic patients had significantly higher sST2 serum concentrations as depicted in **Figure 4.** The authors therefore concluded that sST2 serves as a potent marker for Th2 cytokine producing cells and therefore provides further evidence for a shift from Th1- to Th2-biased cells.[98]

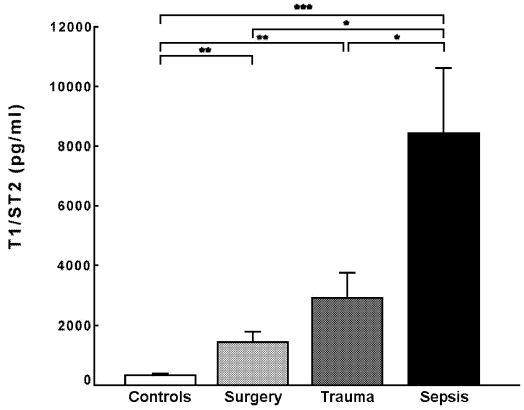

Figure 4: Increased sST2 serum concentrations in septic patients.
ST2 serum concentrations of patients diagnosed with sepsis, trauma, patients undergoing abdominal surgery and 15 healthy subjects. Adapted from Brunner and colleagues.[98]

On-versus off pump CABG surgery

Since the first open cardiac surgery in the 1950s CPB remains a major contributor to early mortality and morbidity after heart operations.[99] The transient introduction of synthetic non-degradable materials of the heart-lung machine is known to induce an immune response.[99] The particular type of immune response is determined by differentiation of precursor T helper (TH0) cells into Th1 or Th2 subsets.[100] In 2005 Ankersmit and his study group reported that CBP utilization during coronary artery bypass graft (CABG) surgery leads to an enhanced Th2 cytokine production.[101] In this study on 30 patients undergoing on- or off-pump CABG, soluble (sST2) and interleukin (IL)-10 serum concentrations were measured prior to surgery and 30min, 60 min and 24hrs thereafter. In this study, sST2 raised 400-500 fold within 24 hours after surgery irrespective of the surgical procedure. In contrast to sST2, IL-10 rose statistically significant in the on- pump compared to the off pump group as shown in **Figure 5**.[101]

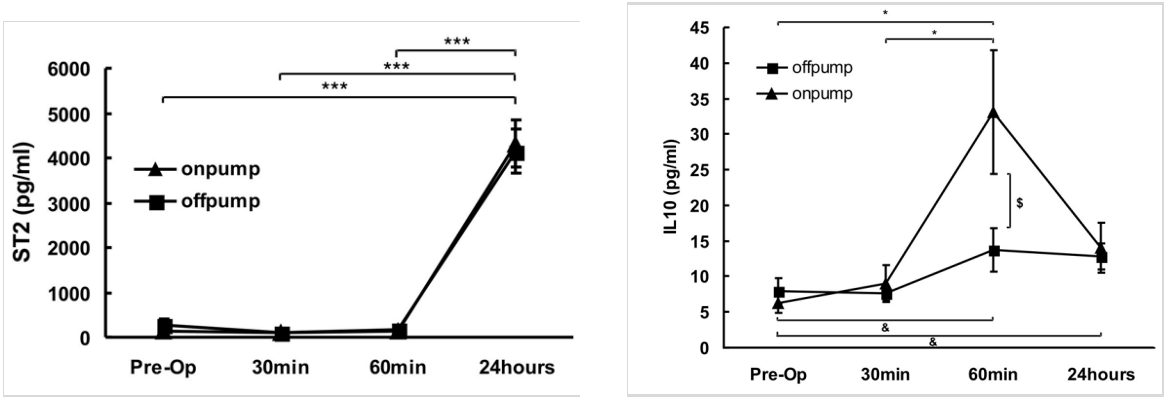

Figure 5: Serum concentrations of sST2 and IL-10 during the CABG procedure.
Serum concentrations of on-pump and off-pump patients were measured before surgery and 30, 60 minutes, and 24 hours after surgery. Adapted from Szerafin and colleagues. [101]

Four years thereafter Ankersmit and his co-workers investigated again the perioperative inflammatory reaction after heart surgery on CPB, but more extensively. Back then, data on the prolonged postoperative time course of the triggered immune reaction was not available. Therefore, his study group investigated in 16 patients after CABG on CPB the time course of inflammatory (TH1) and anti-inflammatory cytokines (TH2) in a sequential fashion until day eight. In this investigation, a marginal elevation of pro-inflammatory and an extensive increase of anti-inflammatory cytokines was reported as depicted in **Figure 6**. Supporting a clear TH2 overexpression after CABG surgery that may explain why patients after CABG surgery are more susceptible to local and systemic infection.[87]

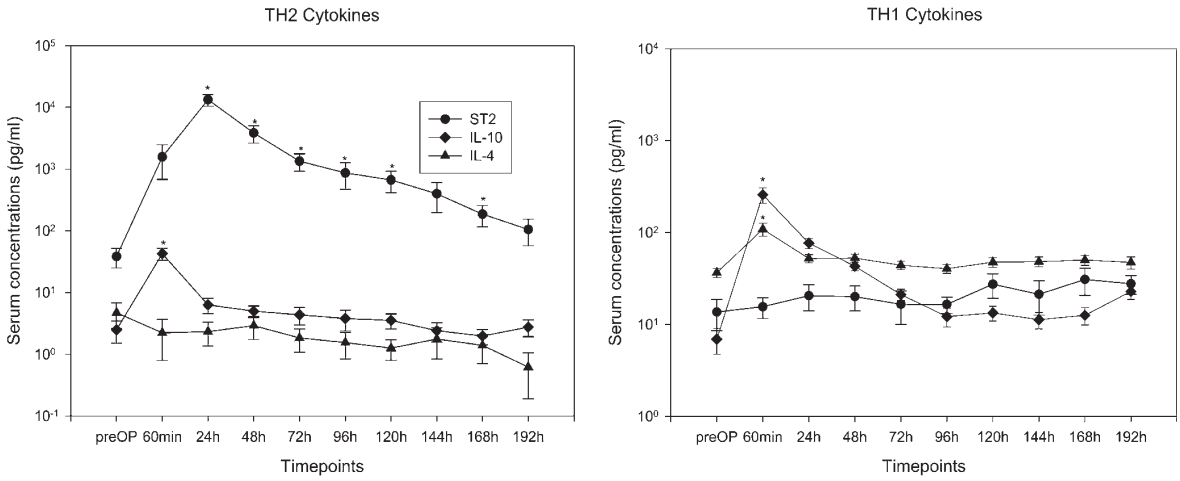

Figure 6: Serum concentration of TH2 and TH1 cytokines.

ST2, IL-10 and IL-4 serum concentrations of patients undergoing CABG surgery on CPB (left). IFN-gamma, IL-6 and IL-8 serum concentrations of patients undergoing CABG on CPB (right). Adapted from Szerafin and colleagues.[87]

Continued mechanical ventilation during CPB

Continued airway ventilation is commonly discontinued during CPB in cardiac surgery. Iatrogenic atelectasis is well accepted in order to guarantee better visualization of the operative field. The dual oxygen supply of the lung is thus solely maintained through the bronchial arteries with a potential rise of pulmonary mal-perfusion, increasing the risk of adverse pulmonary outcome.[102] To get a better picture of the effect of atelectasis during CPB on inflammation Beer et al. analyzed for the first time in 2013 the systemic anti- and pro-inflammatory response of 30 randomized patients undergoing either conventional CABG surgery with continuous ventilation on CPB (ventilated; n = 15) or no ventilation on CPB (non-ventilated; n = 15). Cytokine serum concentrations of sST2, IL-4, IL-10, IgM, IgG, IL-6 and endotoxin were measured via ELISA testing. As a main result of this study, Beer and colleagues found significantly reduced sST2 serum concentrations in ventilated patients compared to non-ventilated patients at postoperative day (POD)-1, POD-2, POD-3 and POD-5. Further, IL-10 and IL-6 concentrations were significantly lower in ventilated patients at POD-1 when compared to non-ventilated patients.[103]

Soon thereafter the same study group investigated the perioperative serum concentration of stress inducible heat shock proteins (HSP)s in ventilated and non-ventilated patients during CABG surgery on CPB. During cellular injury, regulation of

inflammatory responses and tissue repair stress inducible HSPs play an important role.[104] HSPs have both: immune stimulatory and immune suppressive functions depending on their molecular weight and their localization.[105] It has been found that during continued mechanical ventilation on CPB for CABG surgery HSP70 serum concentrations were markedly reduced after surgery. However, HSP27, HSP60 and apoptosis markers were not lowered by continued mechanical ventilation.[106] In the same year the same study group published the impact of continued mechanical ventilation on the expression of pro-inflammatory chemokines (CCL2, CCL4, and CCL20).[107] Chemokines are known as small proteins that can be subdivided into four groups according to the position of the N-terminal cysteine in the protein conformation (CC, CXC, C, and CX3C). Chemokines regulate leucocyte trafficking and are released during cardiac surgery.[108, 109] Especially, CCL2 and CCL4 are important, since both chemokines are secreted from alveolar macrophages during alveolar hypoxia and during endotoxemia.[110] Furthermore, chemokines, pro-inflammatory cytokines and adhesion molecules, aggravate the development of LPS induced acute lung injury.[110] Beer and colleagues found that heart surgery on CPB induced a systemic release of CCL2, CCL4, and CCL20. Moreover, they found that continued mechanical ventilation during CPB significantly reduced CCL4 serum concentrations on the first 5 PODs and slightly attenuated CCL2 and CCL20 release postoperatively.[107] In 2015 Beer et al. published the final work on continued mechanical ventilation during CPB. In this study the investigated MMPs, TIMP1, and LCN2 in the context of CPB and ventilation was investigated.[111] MMPs are zinc- and calcium-dependent endopeptidases and are important during protein degradation of the extracellular matrix and during tissue remodeling. Furthermore, MMPs support leukocyte extravasation and therefore amplify local immune reactions.[112] Lin and colleagues reported that MMP serum concenrations were increased in patients undergoing cardiac surgery.[113] Another study found that MMP-9 serum concentrations rose on a lower level in patients undergoing off-pump CABG surgery in contrast to patients undergoing on-pump surgery.[114] Lipocalin 2 (LCN2), known as a small protein that is highly expressed during infections, tissue and I/R injury builds complexes with MMP-9, thereby stabilizing MMP-9 activity.[117]

Beer and colleagues found that MMP-8, MMP-9, and LCN2 serum concentrations were significantly lower after surgery in continuously ventilated patients during CPB compared to non-ventilated patients. Further, they found that TIMP-1 concentrations were lower on POD 1 and MMP-3 levels were lower on POD 4 and 5.[111] According to the findings of Beer and colleagues in these 4 studies it can be concluded that continued mechanical

ventilation during CPB in patients undergoing CABG surgery appears advantageous to avoid postoperative pulmonary dysfunction.

Until now, two large randomized trials investigated whether continued low tidal mechanical ventilation has impact on postoperative outcome: In 2019 the PROVECS randomized, multicenter, controlled trial assigned 493 patients to either no or maintained mechanical ventilation during CPB. The primary end point of this study was a composite of respiratory complications occurring within the first 7 days after surgery. Postoperative respiratory complications occurred in 54.7% of all patients who were assigned to maintained mechanical ventilation and in 59.2% of all patients who were assigned to discontinued mechanical ventilation. While absolute values of this trial seem to point toward a better outcome for patients receiving maintained ventilation, there was no significant difference between both groups.[118] More recently, in 2021 the MECANO trial, published in *the CHEST* randomized 1051 patients in a single center to continued or discontinued mechanical ventilation during CPB. In this study they found no statistically significant difference among both groups regarding the incidence of a composite outcome of death, early respiratory failure, ventilation support beyond day 2, and re-intubation. However, in a subgroup analysis of patients undergoing isolated CABG the continued mechanical ventilation was significantly associated with a better primary outcome.[119]

Inflammation and left ventricular assist devices

In 1999 Ankersmit investigated at the Columbia University of New York the association between left ventricular assist device (LVAD) related infections and host immunity in 78 patients with New York heart association class IV who received either an LVAD (n=40) or medical therapy (controls, n=38). At that time, long-term outcome after LVAD implantation was known as limited due to tremendously high rates of opportunistic infections.[121] In this study, Ankersmit and colleagues found that 3 months after LVAD implantation 28% of all LVAD recipients and 3% of all patients receiving medical therapy developed candida infections. Moreover, LVAD recipients had cutaneous anergy to recall antigens and lower T-cell proliferative responses after activation via the T-cell receptor complex. Additionally, T cells from patients with an implanted LVAD had a higher surface expression of CD95 and a significantly increased rate of spontaneous apoptosis than patients receiving solely medical therapy. Ankersmit and his colleagues concluded, as published in the LANCET that LVAD implantation results in aberrant T-cell activation, increased susceptibility of CD4 T cells to undergo activation-induced cell death and immune cell deficiency which may lead to opportunistic infection.[122] Soon thereafter, Ankersmit performed another

analysis on LVAD recipients and demonstrated that these patients had a relative lymphopenia and reduced CD4 T-cell levels compared to patients with heart failure receiving medical therapy only. Further, they found in a longitudinal analysis that LVAD implantation was accompanied by a progressive and long-lasting reduction of circulating CD4 T-cell levels. These findings were accompanied by excessive T-cell apoptosis and increased levels of circulating soluble CD95, which is known as a marker for increased T-cell apoptosis. Suggesting that reduced CD4 T-cell levels in LVAD recipients may be a consequence of an augmented pathway of CD95-mediated apoptosis which is clinically tangible as a state of reduced immune competency.[123] More recently, 20 years later, Opfermann and co-workers investigated the profile of sST2 production after LVAD insertion in severely ill patients with end-stage heart failure. The study group found extremely high sST2 serum concentrations after LVAD implantation with a maximum peak above 400 ng/ml at day 1. Furthermore, Opfermann et al. compared survivors and non-survivors and found persistently elevated sST2 levels in non-survivors during hospital stay as depicted in **Figure 7.**[124]

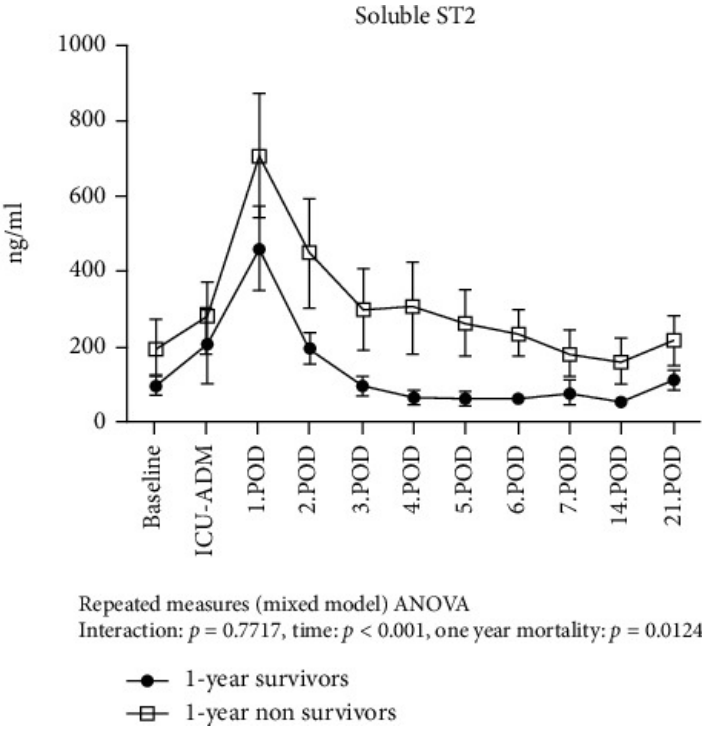

Figure 7: Longitudinal changes of sST2 serum concentrations of LVAD patients.

The longitudinal curse of sST2 serum concentrations of 1-year survivors and 1-year non-survivors. Adapted from Opfermann and colleagues.[124]

1.1.4 SIRS, qSOFA and SOFA

SIRS

The term sepsis was introduced for the very first time by the consensus conference of sepsis in 1991. Back then, sepsis was thought to occur only as result of infection. To establish the diagnosis of Sepsis as a result of infection in conjunction with hyper-inflammation four SIRS criteria were established: tachypnea, with a respiratory rate above 20 breaths per minute, tachycardia, with a heart rate above 90 beats per minute, leukopenia or leukocytosis with a leucocyte count above 12,000 cells/µL or below 4000/µL and fever or hypothermia with a body temperature above 38 °C or below 36 °C. [34, 125] Sepsis in conjunction with organ dysfunction was defined as severe sepsis. Severe sepsis together with persisting sepsis-induced hypotension, despite adequate fluid resuscitation was defined as septic shock.[34, 125]

SOFA

Soon thereafter, SOFA was introduced as a scoring system to quantify organ dysfunction in ICU patients.[126] The application of this scoring system widely expanded over time and it is now applied as a key criterion in the diagnosis of sepsis syndrome.[127] Recently, sepsis syndrome was redefined as a life-threatening organ dysfunction caused by an imbalanced immune system in response to sterile or non-sterile infection.[127] An increase of more than two points of SOFA compared to baseline levels was reported to be associated with a mortality rate greater than 10%. Details of the SOFA are shown in **Table 1**. Furthermore, Septic shock was redefined as sepsis in conjunction with circulatory, cellular and metabolic abnormalities according to following criteria: vasopressor support to maintain a mean arterial pressure of 65 mmHg and a serum lactate level of more than 2 mmol/L in the absence of hypovolemia. The combination of both was reported to be associated with a hospital mortality rate greater than 40%.[127]

qSOFA

The quick (qSOFA) was established as an easy applicable bedside scoring system to identify patients with suspected infection or sepsis in the general hospital wards. This bedside tool monitors respiratory, neurologic and hemodynamic function as following:

respiratory rate of 22/min or greater, altered mentation, or systolic blood pressure of 100 mm Hg or less. [128] [127]

Table 1: Sequential Organ Failure Assessment Score.

SOFA assesses 6 organ systems in ICU patients. Adapted from Singer and colleagues, published in 2016.[127]

	Score				
System	0	1	2	3	4
Respiration					
Pao_2/Fio_2, mm Hg (kPa)	≥400 (53.3)	<400 (53.3)	<300 (40)	<200 (26.7) with respiratory support	<100 (13.3) with respiratory support
Coagulation					
Platelets, $\times 10^3/\mu L$	≥150	<150	<100	<50	<20
Liver					
Bilirubin, mg/dL (μmol/L)	<1.2 (20)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	>12.0 (204)
Cardiovascular	MAP ≥70 mm Hg	MAP <70 mm Hg	Dopamine <5 or dobutamine (any dose)[b]	Dopamine 5.1-15 or epinephrine ≤0.1 or norepinephrine ≤0.1[b]	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1[b]
Central nervous system					
Glasgow Coma Scale score[c]	15	13-14	10-12	6-9	<6
Renal					
Creatinine, mg/dL (μmol/L)	<1.2 (110)	1.2-1.9 (110-170)	2.0-3.4 (171-299)	3.5-4.9 (300-440)	>5.0 (440)
Urine output, mL/d				<500	<200

FIO_2 fraction of inspired oxygen; *MAP* mean arterial pressure; *PaO_2* partial pressure

1.2 Extracorporeal membrane oxygenation

John Gibbon became first by inventing the heart-lung machine in the 1930's in the General Hospital of Massachusetts. His wife, Mary Gibbon was the first perfusionist. Twenty years later, in 1953, John Gibbon performed the first successful cardiac procedure on CPB in humans, by closing an atrial septum defect in an 18-year-old woman.[129] In Austria, the first successful heart surgery on CPB was performed in Graz in 1962. Since then, the conduct of the CPB has been steadily improved over the years.[130] In contrast to CPB, Extracorporeal membrane oxygenation (ECMO) presents an option for prolonged treatment of refractory cardiovascular and respiratory failure outside the operating room. During ECMO support, blood is drained from the native vascular system, circulates by a mechanical pump through an oxygenator and heat exchanger. The oxygenator saturates haemoglobin fully with oxygen, while carbon dioxide (CO_2) is removed. Blood flow rates control adequate oxygenation. In contrast, CO_2 elimination can be adjusted by the counter current gas flow through the oxygenator.[131] There are two types of ECMO support systems comprising venoarterial (va) and venovenous (vv) canulation.[132]

1.2.1 ECMO systems: Design and variants

Vv ECMO

Vv support systems solely work as respiratory support systems by connecting an artificial lung and the natural lung in series for gas exchange. Since, hemodynamics are not supported by vv ECMO systems, sufficient cardiac function is required to guarantee an adequate systemic blood flow and blood pressure.[133] There are two different variants for placing the cannulas: femoroatrial or femorofemoral. In both variants, the venous outflow cannula must be placed transfemorally into the inferior vena cava below the branches of the hepatic veins. The inflow cannula returns the blood either through the superior vena cava or the distal inferior vena cava through the ipsilateral or contralateral femoral vein into the right atrium. In both techniques the tip of the inflow cannula must point in the direction of the tricuspid valve to avoid recirculation of the blood through the extracorporeal circuit.[134] Furthermore, double lumen cannula can be placed through the internal jugular vein and the right atrium with the tip pointing into the inferior vena cava.[135]

Rescue high-flow vv ECMO

Oxygen delivery over the artificial membrane is primarily adjusted over the extracorporeal blood flow.[133] High-flow vv ECMO is therefore the intervention of choice for patients with severe hypoxia due to respiratory failure. Since hypoxemia cannot be defined by a single value of PaO_2, hypoxemia is known as a clinical judgment. In the first randomized controlled trial by Warren Zapol in 1979 following thresholds were considered as entry criteria for high-flow ECMO: PaO_2 < 50 mmHg for at least 2 h during 100% FIO_2 and 5 cmH_2O of PEEP or PaO_2 < 50 mmHg for at least 12 h during 60% FIO_2 and 5 cmH_2O of PEEP.[136] The Berlin criteria on the acute respiratory distress syndrome (ARDS) recommend initiating ECMO therapy in patients with severe respiratory failure with a ratio of arterial oxygen tension to fraction of inspired oxygen (PaO2/FiO2) below 70 mmHg.[137]

Low-flow extracorporeal CO_2 removal

In the seventies Kolobow and Gattinoni became first to describe the concept of extracorporeal CO_2 removal ($ECCO_2R$) by developing an arterio-venous pumpless circuit in lambs. Adequate CO_2 elimination was possible at a blood flow below 1000 ml/min equaling around 70ml/kg.[138, 139] Gattinoni and his group introduced in 1986 the concept of "lung rest" in 43 ARDS patients by combining low-flow vv $ECCO_2R$ with protective mechanical ventilation by decreasing respiratory rates and driving pressure and therefore avoiding ventilator-induced lung injuries [140 141 142] The definition of low blood flow ranges from 300 to 400 ml/min up to 1000–1500 ml/ min. In this range the CO_2 clearance, relative to the metabolic production may range from 20 up to 100% depending on input CO_2, membrane lung surface and sweep gas flow.[143] Raised ECMO blood flow induces both: increased oxygenation and decarboxylation, however decarboxylation increases less efficiently above 1500 and 2000ml/min of blood flow.[144] As a result in vv ECMOs with an increased blood flow decarboxylation has to be regulated by adjusting sweep gas flow.[131] During low-flow ECMO oxygenation is limited by the blood flow, since oxygen delivery is at 30% at 1500 ml/min and negligible at 300–400 ml/min of extracorporeal blood flow.[145]

Va ECMO

Va ECMO was initially developed for cardiac surgery; technical developments such as pump miniaturization, better circuit biocompatibility and improved cannulation techniques enabled va ECMO prolongation into the postoperative period.[146] Va ECMO systems were divided into circuits using a central or peripheral vascular access. The peripheral va

ECMO configuration drains blood from the femoral vain and re-infuses the blood through a femoral arterial cannula; thereby generating a retrograde flow up into the aorta that may encounter the anterograde flow generated by the left ventricle. This competition may lead to complications such as left ventricle overload or the Harlequin syndrome depended on cardiac function.[147] The Harlequin syndrome is known as a selective upper body hypoxia occurring in patients with respiratory failure in conjunction with residual heart function; when the watershed between the native poorly oxygenated anterograde blood flow and the well oxygenated retrograde blood flow (assisted by the ECMO) is located below the supra-aortic trunks. Patients developing a Harlequin syndrome show a typical clinical picture of cyanosis in the upper, but not lower part of the body. Accordingly these patients have an increased risk of cerebral or myocardial ischemia. Therapeutic strategies include va ECMO withdrawal, increasing va ECMO blood flow to reduce the native hypoxemic blood flow fraction, or switching to a vv ECMO support if respiratory, but no circulatory support is requested.[148] In addition, a reperfusion catheter must be placed into the ipsilateral superficial femoral artery, since the femeroaterial cannula can lead to obstruction and thereby inducing lower limp ischemia.[146, 149] Central va ECMO cannulation is always a surgical procedure. The venous cannula is inserted in the right atrium and the arterial cannula is placed into the ascending aorta. Since the oxygenated blood is injected anterograde into the aorta, there is no risk of inducing a harlequin syndrome. Central va ECMO is typically considered in patients with post- cardiotomy related heart failure.[150]

1.2.2 ECMO indications for lung transplantation

In 1999 Ko and his working group described the very first application of ECMO support during single lung transplantation (LUTX) in patients with end stage primary pulmonary hypertension.[151] Our institution reported in 2001 for the first time of successful ECMO implantation in a patient with graft failure after cardiac transplantation, when weaning from CPB was impossible.[152] In 2007 our institution presented the first larger case series of patients undergoing double LUTX (DLUTX) on ECMO.[153] Since then, several centers changed their transplantation strategies toward the integration of ECMO support instead of CPB during DLUTX. [154, 155]

ECMO bridging to transplantation

Historically, mechanical ventilation was used as a last bridging option for lung transplant candidates with rapidly advancing end-stage pulmonary disease.[156] In the last decades, ECMO bridging was introduced as an extended rescue therapy in respiratory unstable

patients despite of high respiratory and pharmacologic support.[157, 158] Recently, new ECMO devices with advanced technology were employed to replace mechanical ventilation in lung transplant candidates with the advantage of keeping patients awake and therefore avoiding muscular deconditioning. Therefore, ECMO bridging is currently considered superior to mechanical ventilation.[159] However, bridged patients have an increased risk for ECMO-related complications such as bleeding and thromboembolism. In our institution, Benazzo and colleagues reported a median bridging time of 5 days since Eurotransplant prioritizes patients on extracorporeal life support (ECLS).[160]

Intraoperative ECMO

Intraoperative ECMO support has been integrated as a standard procedure during LUTX at our institution due to following advantages: It combines the benefit of enabling hemodynamic stability and protective low-volume ventilation and further reduces the circulatory blood volume passing the pulmonary vascular bed during the reperfusion period.[161] As a consequence intraoperative ECMO support assists the newly implanted allograft to recover and prevents the development of the first lung syndrome and primary graft dysfunction (PGD). Since the optimal length of reperfusion remains undefined, prolongation of controlled reperfusion into the early postoperative phase via peripheral va ECMO is beneficial in complex or intraoperatively unstable patients.[162]

Postoperative ECMO

To perform ECMO prolongation into the postoperative period after DLUTX there are still on-going discussions concerning superiority of va ECMO over the vv ECMO mode.[163] At our institution the va ECMO mode remains the preferred system due to concomitant hemodynamic stabilization. The decision to perform ECMO prolongation has to be performed according to following predefined quality criteria of patient's respiratory function after central ECMO cross-clamping such as PaO_2/FiO2<100 mmHg, mPAP/mSAP > 2/3, insufficient tidal volumes and a trend of worsening in two consecutive arterial blood gas analyses. [164] If the mentioned criteria were fulfilled the same ECMO system has to be reinserted peripherally into the femoro-femoral va configuration.[165] At the ICU, the need of va ECMO support has to be revaluated on a daily basis. Va ECMO support has to be discontinued if blood flow rates can be reduced to 1.5 L/min without significant hemodynamic or respiratory impairments for at least 4 hours.[162]

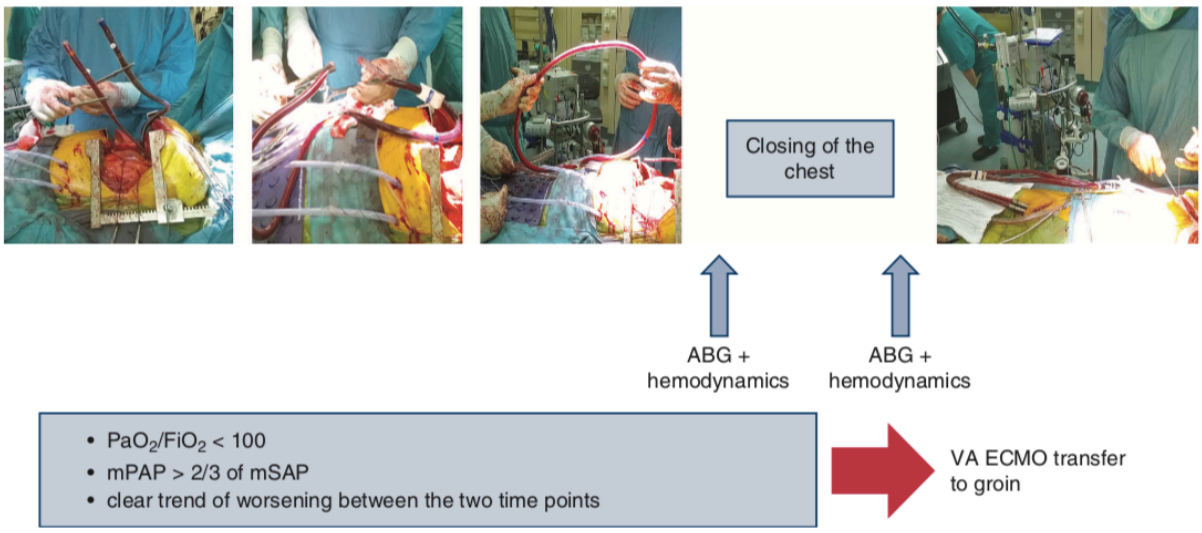

Figure 8: Institutional criteria for ECMO prolongation into the early postoperative period.

If patients do not achieve the mentioned criteria, ECMO will be transferred into the groin. PaO2 Partial arterial pressure of oxygen; FiO2 fraction of inspired oxygen; mPAP mean pulmonary arterial pressure; *mPAP* mean systemic arterial pressure; *ABG* arterial blood gas analysis; *VA* venoarterial; *ECMO* extracorporeal membrane oxygenation. Adapted from Hoetzenecker and colleagues. [165]

Differences between ECMO and CPB according to SIRS

ECMO and CPB are closely related hemodynamic and respiratory supporting systems. Both devices induce an inflammatory response; nevertheless important distinctions between ECMO and CPB exist.[166] The main distinction between ECMO and CPB concerns the duration of hemodynamic and respiratory support provided. While CPB is only employed for minutes to hours to enable cardiothoracic procedures, ECMO can be used for hours, weeks or even months. Further, anticoagulatory strategies differ between both devices; while CPB requires full heparinisation with a loading dose of unfractionated heparin of 300- 500 U/kg, ECMO support is already possible with a loading dose of 40–80 U/kg.[167, 168] Further, after CPB, but not after ECMO support protamine has to be administered to antagonize the effects of heparin. Protamine and heparin can form complexes that are known to exacerbate the inflammatory response via activation of the complement system.[166, 169] In addition, the surgical performance of CPB employment comprises a more invasive surgical strategy comprising cardiotomy, suctioning, venting of blood and the incorporation of venous reservoirs into circuits, introducing blood-air interfaces. Several studies found higher levels of pro-inflammatory cytokines in cardiotomy- suctioned blood. Therefore, the absence of an air/blood interface during ECMO may has inflammatory advantages. [170, 171] Further, perfusion during CPB is non-pulsatile.[172] Several studies suggested that pulsatile blood flow during ECC may reduce the inflammatory response.[173] Last CPB, but not ECMO requires clamping of the aorta, which renders the heart and lungs ischemic. After clamp opening both organs undergo reperfusion. Unfortunately, ischemia and reperfusion induce an inflammatory response.[174]

1.3 Lung transplantation and major thoracic surgery

1.3.1 Lung Transplantation

LUTX is defined as the partial or total surgical replacement of a patient's diseased lung. Different types of transplantation are possible such as lobar, single-or double –lung replacement. LUTX in humans was successfully performed for the first time by the Toronto Lung Transplant Group in 1983 thanks to the introduction of cyclosporine for immunosuppression.[175] Initially, LUTX was performed via a single tracheal anastomosis. However, due to high complication rates, sequential bilateral LUTX with two anastomoses on each primary bronchus was introduced soon thereafter.[176]

For candidates waiting for LUTX improved quality of life and prolonged life expectancy after transplantation must be guaranteed after surgery. Since the amount of donor organs remains limited, the allocation and selection of lung transplantation candidates has to follow strict guidelines.[177] Therefore, the Lung Allocation Score (LAS) was developed, with the purpose to properly allocate donor organs and rank patients by the need of urgency for transplantation.[178] The LAS was implemented in the USA in 2005 to replace the ancient scheme, which was solely based on waiting time. The scoring system aimed to lower the number of deaths of patients on the waiting list for LUTX, improving survival of allograft recipients and making the allocation process more efficient and transparent.[178] Germany adopted the LAS already in 2011 and the Netherlands followed two years later, in 2014.[179] For calculating LAS various objective clinical parameters were included to evaluate patient's current health status and to estimate survival probability and predicted 1-year survival rate with or without lung transplantation. Candidates with higher LAS, ranging from 0 to 100, have an increased priority to receive an organ due to an increased probability to benefit in terms of survival. [178-180]

Complications after Lung transplantation

Primary graft dysfunction

PGD occurs in 10 to 30% of all lung transplant recipients and is known as a major limiting factor of early morbidity and mortality after LUTX.[181, 182] In the initial consensus definition of 2005 PGD grading was based on the PaO_2/FiO_2 ratio in the presence of infiltrates in the transplanted lung(s). Grading has to be performed at time point 0 (within 6 hours after reperfusion), 24, 48, and 72 hours after reperfusion of the final lung. In addition, patients on mechanical ventilation with FiO_2 greater than 0.5 or inhaled nitric oxide (iNO) beyond 48 hours and patients on ECLS were considered as PGD grade 3.[183] In 2017 the

consensus statement on PGD was further extended: Transplant recipients on ECMO for hypoxemia with radiographic findings remained as PGD grade 3, patients on ECMO for other reasons without infiltrates confirmed via x-ray were classified as PGD "ungreadable" as depicted in **Table 2**.[184] Recipient-, donor specific and intraoperative risk factors for PGD were shown in **Table 3.**[60] PGD is associated with an increased 90-day mortality rate of almost 40%.[185, 186] Furthermore, PGD is associated with adverse late-term outcome such as CLAD and a reduced quality of life: Fewer patients demonstrate normal 6-minute-walk test one year after LUTX and patients with PGD develop more often delirium post surgery, which is associated with long-term neurological dysfunction. [187,188, 189 190, 191] Until now, there are no treatment options available to reverse PGD. Therefore, supportive care remains the gold standard to treat PGD by performing protective ventilation, administer iNO or epoprostenol and to enhance early mobilization.[192] Besides, ECMO support within 24h after transplantation improved outcomes of PGD patients. Taking into account that patients on ECMO support for PGD had a decreased long-term survival of 65, 40 and 25% for 30-day, 1- and 5- year survival compared to patients without PGD in conjunction with ECMO support.[163, 193] Re-transplantation remains the ultimate therapeutic option for patients with PGD. Nevertheless, studies do not support this option due to a very poor outcome, with a 30- day survival rate of only 31%.[194]

Table 2: PGD grading of the International Society of Heart and Lung Transplantation (ISHLT).

Definition of 2016, adapted from Snell and colleagues.[184]

Grade	Pulmonary edema on chest X-ray	PaO2/FiO2 Ratio	ECLS
0	No	Any	no
1	Yes	> 300	no
2	Yes	200 – 300	no
3	Yes	< 200	no/yes*
"ungradable"	No		yes#

***** Indication for ECLS is hypoxemia, # non-hypoxic indications for ECLS

According to the ISHLT, patients with ECLS employment with bilateral pulmonary edema on chest X-ray should be considered as grade 3. ECLS employment in patients with non-hypoxic indications and no pulmonary edema in the chest X-ray should be classified as "ungradable".

ECLS extracorporeal lung support; *FIO2* fraction of inspired oxygen; *PaO2* partial pressure of arterial oxygen; *PGD* primary graft dysfunction

Table 3: Donor, recipient and operative risk factors for the development of PGD.
Adapted from Shah and colleagues.[192]

Category	Risk factors	Details of PGD association
Donor		
	Mode of death	Traumatic brain injury
		Donation after circulatory death
	Age	Increased age
	Race	African-American race
	Gender	Female gender
	Tabacco use	
	Alcohol use	
Recipient		
	Race	African-American race
	Gender	Female gender
	Pulmonary diagnosis	IPF, sarcoidosis, IPAH
	Pulmonary artery pressure	Increased pulmonary pressure
	Diastolic dysfunction	Left ventricular diastolic dysfunction
	Obesity	
Operative		
	Prior pleurodesis	
	Transplant type	Single lung transplant
	CPB	
	Ischemic time	Longer ischemic time
	Transfusion	Larger volume of transfusion
	DCC	
	Reperfusion FiO_2	Increased reperfusion FiO_2

CPB cardiopulmonary bypass, *DCC* delayed chest closure, *FiO2* fraction of inspired oxygen, *IPAH* idiopathic pulmonary arterial hypertension, *IPF* idiopathic pulmonary fibrosis, *PGD* primary graft dysfunction

Lung transplant rejection

Lung transplant rejection can be categorized into three following subtypes: Hyperacute allograft rejection (HAR), occurring within the first 24 hours, acute allograft rejection which is diagnosed between the first week and the first year and CLAD occurring after the first year post transplantation as depicted in **Figure 9**. [164,195]

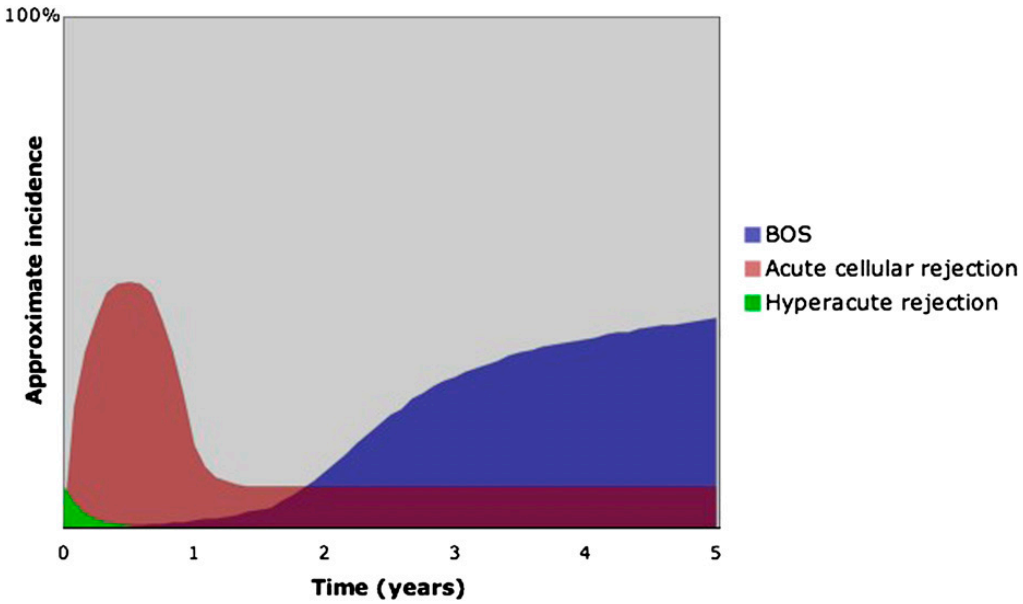

Figure 9: Occurrence of rejection by time post lung transplantation.

Adopted from Martinu and colleagues. [196]

BOS bronchiolitis obliterans syndrome

Hyperacute allograft rejection

Pre-formed antibodies in the recipient directed against the leukocyte antigen (HLA) of the donor causes HAR within the first 24 hours after transplantation.[197] The clinical picture is similar to that of ARDS, with a rapid onset of dyspnea and severe hypoxia caused by acute pulmonary edema and diffuses alveolar damage. HAR is known to carry a extremely high mortality rate.[196, 198] Therefore, performing chest x-ray imaging is important to identify diffuse opacities in the transplanted lung. Further, the diagnosis can be established via immunologic testing for HLA antibodies and reviewing pre-transplant virtual crossmatch results. Exclusion of differential diagnoses must be performed for establishing the diagnosis for HAR.[199]

Acute Allograft rejection

According to the registry of the ISHLT 28% of all LUTX recipients have at least one episode of treated acute rejection in the first year after the procedure.[200] Acute allograft rejection can be further classified into T-lymphocyte-mediated acute cellular rejection (ACR), and antibody-mediated reaction (AMR) directed against major HLAs.[196]

Acute cellular rejection

The diagnosis of ACR can be established by excluding other infections, especially cytomegalovirus in conjunction with histopathologic findings in transbronchial biopsies obtained via bronchoscopy. Histopathologic findings can be present in the vasculature and the airways. Therefore, ACR can be further divided into acute rejection, concerning the vasculature or as lymphocytic bronchiolitis affecting the airways. [201-203] At least five pieces of alveolated lung parenchyma are recommended for the assessment of ACR. [201] The clinical picture of ACR ranges from no symptoms to fever, cough, dyspnoea, or even severe ARDS.[204] ACR is one of the main risk factors for LUTX patients to develop bronchiolitis obliterans syndrome (BOS), the major form of CLAD. Increased severity of rejection and number of episodes of rejection increase the risk of BOS.[205, 206, 207] Therefore, early detection and treatment of ACR is important. Nevertheless, performing surveillance bronchoscopy to screen asymptomatic patients for ACR remains controversial. In the US 69% of all lung transplant centers perform surveillance bronchoscopies on a regular basis.[208] Treatment is generally performed with pulse corticosteroids, such as intravenous methylprednisolone of 10–15 mg/kg daily or 500 to 1,000 mg daily for 3 days. An oral prednisone taper may follow.[209]

Acute antibody mediated rejection

AMR is a well-recognized entity following heart and kidney transplantation and is known as the second phenotype of acute allograft rejection. Donor-specific antibodies DSA are either present prior to transplantation or emerge as de novo DSA (dn)DSA. [209, 210] AMR develops due to DSAs binding to foreign HLA or other donor epitopes of the newly transplanted lung and thereby stimulating immune responses either complement-dependent or by directly activating immune cells such as macrophages or natural killer cells.[210, 211] AMR can be divided into a clinical and a sub-clinical manifestation form, dependent on the presence of allograft dysfunction.[212] AMR is then further sub-categorized into definite, probable, and possible based on the number of diagnostic criteria.[212] Since symptoms can be absent, it remains hard to establish the diagnosis of AMR.[212, 213] Symptoms of AMR comprise dyspnea, fatigue, decreased oxygen saturation, decline in FEV1 and signs in radiologic examination. To establish the diagnosis of AMR histological features of AMR, C4d staining and DSA have to be evaluated.[214] Until now there is no standardized treatment regimen for AMR available, since randomized trials for AMR treatment remain outstanding. However, at the moment two different treatment options are available to combat AMR: Circulating DSA can be either reduced via plasmapharesis or by suppressing the function of B and plasma cells to avoid further antibody formation.[215]

Chronic lung allograft dysfunction

According to international data CLAD is known as a main reason for reduced life expectancy after LUTX, occurring in more than 50% of all recipients within 5 years after transplantation.[195, 216, 217] CLAD can be further devised into two phenotypes, comprising BOS and Restrictive Allograft Syndrome (RAS). While Bronchiolitis Obliterans (BO) is a histopathological diagnosis defined as "*narrowing or complete occlusion of the small airways"*, BOS is known as a clinical correlate of BO presenting irreversible airway obstruction. In contrast to BOS, RAS solely contributes to 25–35% of all CLAD patients. BOS has a better average life expectancy compared to RAS of 1200 versus 540 days. Histologically RAS presents diffuse alveolar damage in addition to fibrosis of the alveolar interstitium.[218] In general, the diagnosis of CLAD has to be established by excluding other causes leading to a decline of Forced Expiratory Volume in one second (FEV1). Other causes were can be further divided into *allograft-related* and *extra-allograft-related* factors comprising acute rejection, Azithromycin Responsive Allograft Dysfunction, infection/colonization anastomotic stricture and pleural disease and diaphragmatic

dysfunction.[217, 219]At our institution CLAD rates and overall outcome, beside ACR and AMR, improved dramatically after introduction of Alemtuzumab. Benazzo et al. found in 721 LUTX recipients after 1, 5, and 10 years that freedom from higher-grade ACR was present in 98%, 96%, and 96%, freedom from CLAD occurred in 94%, 72%, and 53% and overall survival rates were at 85%, 71%, and 61%. Besides, over the years 5% of all patients developed clinical AMR.[220]

Immunosuppression after Lung transplantation

Induction therapy

Induction therapy plays a pivotal role to avoid acute allograft rejection and subsequently CLAD.[221] Two different types of induction therapy exist comprising monoclonal and polyclonal lymphocyte-depleting agents. [222]

Lymphocyte-depleting agents

The most famous polyclonal lymphocyte-depleting agent is Antithymocyte globulin (ATG) acting through complement and antibody-mediated cell lysis and opsonization and phagocytosis via macrophages.[223, 224] The most important monoclonal lymphocyte-depleting agent is Alemtuzumab, acting through binding on CD52 of the cell surface of B and T cells, monocytes, macrophages and natural killer cells. [223, 224] At our institution Alemtuzumab is mainly used, since studies found improved freedom from acute allograft rejection, prolonged CLAD-free survival and overall survival compared to ATG.[225, 226]

Maintenance therapy

Maintenance therapy commonly consists out of a triple drug immunosuppression regimen comprising corticosteroids, a cytostatic agent and a calcineurin inhibitor. The mainly prescribed combination regimen according to the ISHLT consists out of tacrolimus, mycophenolate mofetil [227] and corticosteroids. Combining 3 different drugs improve outcome by reducing side effects due to lower target levels. [222, 224]

Corticosteroids

Systemic corticosteroids are key components of modern immunosuppressive therapy after organ transplantation, even though side effects occur frequently. [222, 228] Corticosteroids reduce the inflammatory response by inducing neutrophil leukocytosis and attenuate

circulating eosinophils, monocytes, and lymphocytes. Antibody production remains functioning under systemic corticosteroids, since B-cells remain unaffected.[229]

Cytostatic agents

Mycophenolate mofetil (MMF) is an antimetabolite that blocks the de novo pathways of nucleotide synthesis by inhibiting the purine and/or pyrimidine synthesis. Next to the *de novo* pathway, a salvage pathway also synthesizes nucleotides in many cells excluding lymphocytes. Since lymphocytes solely have the de novo pathway, MMF exerts their anti-proliferative effect on especially on these cells.[228-230]

Calcineurin inhibitors

Tacrolimus is a FK-binding protein in the cytoplasm of T-cells. It is the mainly applied calcineurin inhibitor for maintenance therapy. Tacrolimus inhibits calcineurin from translocating to the nucleus that leads to a shortage of IL-2 mRNA transcription. IL-2 mRNA transcription is important for activation and proliferation of T lymphocytes.[224, 231-233]

1.3.2 Pulmonary Endarterectomy

Pulmonary endarterectomy (PEA), performed in experienced centres is the gold standard to treat chronic thromboembolic pulmonary hypertension (CTEPH).[234, 235] In 2009 Condliffe and colleagues reported that more than 90% of all patients undergoing PEA are cured from CTEPH. However, 32% of all CTEPH patients are judged inoperable and 76% of all untreated CTEPH patients die within 3 years after diagnosis.[236] The current surgical San Diego classification system for CTEPH categorizes patients in four different types (**Figure 10)**:

- **Type 1:** consisting out of a fresh thrombus in the main-lobar pulmonary arteries
- **Type 2:** characterized by intimal thickening and fibrosis in the proximal segmental arteries
- **Type 3:** located within the distal segmental arteries
- **Type 4:** consisting out of distal arteriolar vasculopathy without detectable thromboembolic disorder

Patients diagnosed with Type 1 and 2 are known to benefit the most from PEA, whereas patients with type 3 and 4 have an increased surgical risk and a worse prognosis.[237] PEA includes following surgical steps: median sternotomy, initiation of CPB, deep hypothermia and two series of complete cardiac arrest. The specimen has to be dissected via a proximal intrapericardial pulmonary artery incision. Intraluminal fibrinous and intimal material has to be removed down to the lobar, segmental and subsegmental pulmonary artery branches on each side. After removal of the specimen, patients have to be rewarmed to wean them form CPB.[238, 239]

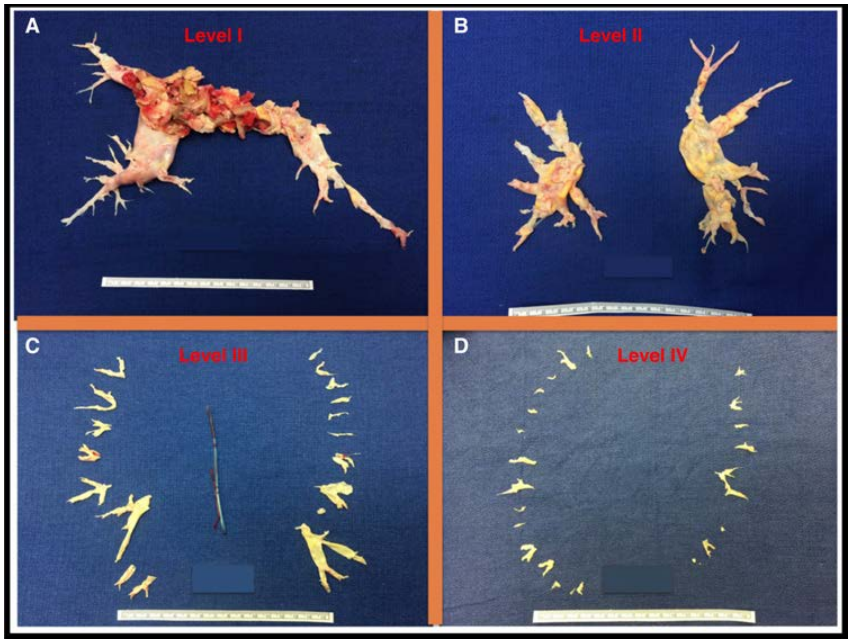

Figure 10: According to the San Diego classification of CTEPH

Surgical specimens according to the level of obstruction from Type 1-4 are depicted above. Adapted from Madani and colleagues. [240]

1.3.3 Lobectomy

Lobectomy is defined as the surgical removal of the entire lobe of the lung. In 1913 Dr. Davies performed a lobectomy for the very first time. However, the patient died within one week after the operation, due to a postoperative infection.[241] Traditionally, lobectomy was performed via thoracotomy. Nowadays, video-assisted thoracoscopic surgery (VATS) has become the procedure of choice.[242, 243] Lobectomy is indicated to resect benign and malignant lung diseases.[241] For patients undergoing lobectomy an adequate pulmonary reserve must persist.[242] Complications after lobectomy depend on patients' diagnosis and the resected lobe.[244] Usually, complications occur in the early postoperative period, within 48 hours.[245] In 2009 a review of the national cancer database in the US found that the mortality and morbidity rate after lobectomy is at 2.6% and 10-50%.[246] Main complications in the early postoperative period are prolonged air leak (15% to 18%), subcutaneous emphysema, pneumonia/mucus plugging/atelectasis (6%), pleural empyema (1% to 3%), persistent space (9.5%) atrial fibrillation (33%), right middle lobe torsion (0.09 to 0.4%), hemorrhage (2.9%), chylothorax (0.7% to 2%), phrenic nerve injury and recurrent laryngeal nerve injury, wound infection, tumor embolization (less than 1%) and rarely bronchopleural fistula.[245]

1.4 Pulmonary disease

1.4.1 Chronic Obstructive Pulmonary Disease

Chronic Obstructive Pulmonary Disease (COPD) is a chronic and progressive condition with airflow limitation provoked by a mixture of small airway disease (obstructive bronchiolitis) and parenchymal destruction resulting in prolonged inflammation of the airways.[247-249] Currently COPD is the fourth leading cause of death of the entire world.[250] The main risk factors for COPD are tobacco smoking, biomass fuel exposure and air pollution. Nevertheless, unavoidable predisposed genetic factors for the development of COPD exist.[251, 252] The best documented, however rarely occurring genetic risk factor is the deficiency of alpha-1 antitrypsina major circulating inhibitor of serine proteases.[253]

Diagnosis of COPD

Patients who show clinical symptoms such as dyspnea (cardinal symptom), chronic cough (first symptom) or sputum production, and/or report a history of exposure must be further examined for COPD via spirometry.[254, 255] Spirometry is a pulmonary function test that enables to measure non-invasively the volume of air forcibly exhaled from the point of maximal inspiration, called the forced vital capacity (FVC) and the volume of air exhaled during the first second, named the forced expiratory volume in one second (FEV_1). The ratio of FEV_1/FVC can be calculated and are evaluated according to reference values based on age, height, sex, and race.[256] COPD patients with reduced airflow measured via spirometry were graduated into four categories according to their (FEV_1/FVC) ratio as shown in **Table 4**.[126]

Table 4: The GOLD classification of airflow limitation severity in COPD patients.
Adapted from Shen and colleauges.[257]

In patients with FEV1/FVC < 0.70		
GOLD 1	mild	$FEV_1 \geq 80\%$ predicted
GOLD 2	moderate	$50\% \leq FEV_1 < 80\%$ predicted
GOLD 3	severe	$30\% \leq FEV_1 < 50\%$ predicted
GOLD 4	very severe	$FEV_1 < 30\%$ predicted

FVC forced vital capacity *FEV_1* Forced expiratory volume in one second, *GOLD* Global Initiative for Chronic Obstructive Lung Disease

Smoking cessation

Smoking cessation impacts dramatically on the improvement of the natural history of COPD.[258] Nicotine replacement therapy is known as a useful tool to increase long-term smoking abstinence rates. Several contraindications for nicotine replacement therapy exist such as recent myocardial infarctions and strokes. [259]

Bronchodilators

Bronchodilators dilate the bronchi and bronchioles by relaxing airway smooth muscles. Accordingly, bronchodilators reduce hyperinflation and improve exercise performance in patients with COPD.[260, 261]

Beta2-agonists relax airway smooth muscles via beta2-adrenergic receptors, which increase cyclic AMP to antagonize bronchoconstriction. Two different types of Beta2-agonsists exist: Short-acting (SABA) (4-6 hours) and long-acting (LABA) (>12 hours). [262 263 264]

Antimuscarinic drugs are anticholinergic agents that block the activity of the muscarinic acetylcholine receptor in airway smooth muscles. Short- and long-acting antimuscarinic antagnosists (SAMAs and LAMAs) exist. [265]

Inhaled corticosteroids

Inhaled corticosteroids (ICS) can improve lung function, health status and reduce the incidence of exacerbations in combination with LABA.[266] However, ICS intake is linked to adverse effects comprising oral candidiasis, hoarse voice, skin bruising, pneumonia, onset of diabetes, poor control of manifested diabetes, cataracts and tuberculosis.[268,269, 270]

Antibiotics

In patients with COPD azithromycin or erythromycin therapy for at least one year reduces the risk of COPD exacerbation in former smokers.[271] However, Azithromycin intake is associated with bacterial resistance and impaired hearing tests.[272]

Interventional therapy options for COPD

Lung volume reduction surgery (LVRS)

LVRS aims to improve mechanical efficiency of respiratory muscles by removing hyper-inflated lung areas. [273-275] This surgical procedure increases the elastic recoil pressure of the lung, resulting in increased expiratory flow rates and thus reducing exacerbations.[276] LVRS can be performed via video- assisted thoracotomy or thoracotomy to remove 20–30% of the emphysematous lung and allows the remaining lung to expand and thereby improving vital capacity, elastic recoil and V/Q matching.[277] Additionally, the reduced residual volume helps the diaphragm to reorient to a normal dome shape and improving respiratory mechanical efficiency.[278]

Endobronchial valves

Endobronchial valves are minimally invasive techniques to achieve similar physiological improvements compared to LVRS. These one-way valves are placed via bronchoscopy, to block inspiratory flow in a lobar bronchus causing lobar atelectasis and therefore relieving dead space ventilation, hyperinflation and improving vital capacity. [279]

Lung transplantation

LUTX has been implemented as a final treatment strategy for appropriately selected patients with very severe COPD. Furthermore, LUTX only improves health status and functional capacity and does not prolong survival.[280]

1.4.2 Idiopathic pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a pathophysiological haemodynamic state, defined by an elevated mean pulmonary arterial pressure (mPAP) above 20 mmHg at rest as assessed by right heart catheterization (RHC).[281] The World health organization [282] classifies PAH according to histological- and pathophysiological patterns into five groups (**Table 5**). Group 1 comprises idiopathic and familial PAH.[283] Group 2 includes PAH resulting from either left ventricular or global heart failure with reduced or preserved ejection fraction (HFrEF) or (HFpEF) and valvular heart disease. Group 3 encompasses PAH secondary to chronic lung diseases and/or hypoxia. Group 4 encompasses CTEPH patients. Group 5 stands for secondary PH syndromes due to heterogeneous systemic diseases such as sarcoidosis, histiocytosis, hematological disorders and extrinsic compression of the pulmonary artery.[284, 285] In 2015 the European Society of Cardiology /European Respiratory Society guidelines determined PAH as present with a mPAP ≥25 mmHg.[286] During the 6th World Symposium on PAH the threshold was reduced from ≥25 mmHg to >20 mmHg due to raising evidence from several research groups on PAH which have shown that patients with mPAP between 21 and 24 mmHg had a higher risk to progress to ≥25 mmHg and a increased mortality rate compared to patients with mPAP <20 mmHg.[287-289]

Idiopathic PAH (IPAH) occurs in 1–3 cases per million people and is therefore determined as an orphan disease with unknown underlying cause.[290] IPAH is known as a clinical syndrome; since diagnosis can only be established by excluding secondary causes and associated conditions.[284] IPAH affects the small distal pulmonary arteries with a diameter less than 500nm, showing hypertrophy of the media, intimal proliferative and fibrotic changes, thickening of the adventitia with perivascular inflammatory infiltrates and thrombotic lesions.[291, 292] Subsequently, these pathologic transformations cause excessive vasoconstriction.[293] Genetic mutations were identified for IPAH.[294]

Table 5: Hemodynamic definitions of pulmonary hypertension (PH).

Adapted from Galiè and Simonneau.[284,295]

Definitions	Characteristics	Clinical groups
Pre-capillary PH	mPAP >20 mmHg PAWP ≤15 mmHg PVR ≥3 WU	1, 3, 4 and 5
Isolated post-capillary PH	mPAP >20 mmHg PAWP ≥15 mmHg PVR <3 WU	2 and 5
Combined post-capillary	mPAP >20 mmHg PAWP ≥15 mmHg PVR ≥3 WU	2 and 5

Group 1 = IPAH; group 2 = PH caused by heart diseases; group 3 = PH caused by lung diseases and/or hypoxia; group 4 = PH caused by pulmonary artery obstructions; group 5 = PH with unclear cause and/or induced by multifactorial mechanisms.

WU were calculated as following: SVR= (MAP-CVP)/CO *80

mPAP mean pulmonary arterial pressure*; PAWP* pulmonary arterial wedge pressure*, PVR* pulmonary vascular resistance*; WU* Wood Units

Medical treatment of IPAH

IPAH treatment targets on three major pathways: the NO, endothelin-1 (ET-1) and the prostacyclin (PGI2) pathway. NO is an endothelium-derived vasoactive mediator increasing the production of cyclic guanosine monophosphate (cGMP) via activation of soluble guanylate cyclase (sGC). NO thereby leads to vasodilation and inhibits proliferation within the vasculature smooth muscle cells.[293, 296] The NO pathway can be targeted by two different drug classes: Phosphodiesterase inhibitors (PDEis) and sGC stimulators. The enzyme phosphodiesterase enhances the conversion of cGMP to GMP. By inhibiting this pathway the cGMP breakdown can be avoided. sGC stimulators directly increase the production of cGMP.[286] ET-1 is a potent vasoconstrictor and plays a pivotal role on pulmonary vascular remodeling by acting on two receptors: The endothelin receptor A (ET_A) and the endothelin receptor B (ET_B).[293] In PAH patients ET-1 is up-regulated and therefore inducing cellular proliferation, fibrosis, inflammatory reactions and vasoconstriction via ET_A and ET_B.[296, 297] Until now there is one drug class: endothelin-receptor antagonists (ERA) which are targeting the ET-1 pathway.[298]

PGI2 is a potent vasodilator by increasing cyclic adenosine monophosphate (cAMP) via the prostaglandin I2 (IP) receptor. Besides, PGI2 inhibits platelets aggregation and has antiproliferative properties.[299] In PAH patients the PGI2 synthesis is supressed, subsequently thromboxane A2 concentrations increase, leading to vasoconstriction and platelet activation.[300] There are two drug classes: PGI2 analogues and IP receptor agonists.[301]

NO-Pathway: Phosphodiesterase type 5 inhibitors (PDE5i)

PDE5is are typically the primary therapy of choice for PAH therapy. Initially, in 1998 PDE-5is were only approved to treat erectile dysfunctions and expanded over the years to PAH treatment strategies due to their actions on PDE5 receptors within the pulmonary vasculature (Sildenafil, Tadalafil).[302,303]

NO-Pathway: Guanylate cyclase stimulator

Riociguat was the only sGC stimulator for PAH-specific therapy for a very long time. In 2021 Vericiguat was introduced as a second sGC stimulator. Both agents are the only approved medical therapeutic options for inoperable or persistent CTEPH.[304, 305] Common adverse effects include headache, dyspepsia, dizziness and hypotension. PDE5i and sGC stimulators must not be combined since both drug classes target the NO pathway.[306]

Endothelin receptor antagonists

Until now, there are three ERAs available which are approved for PAH therapy comprising Ambrisentan, Bosentan and Macitentan. All three drugs have teratogenic effects therefore a negative pregnancy test has to be obtained prior to therapy initiation.[285] Ambrisentan preferentially binds to ET_A. Bosentan binds dually on ET_A and ET_B. Since bosentan induces hepatotoxic side effects, liver function testing should be performed monthly in patients receiving bosentan.[307] Bosentan is the only approved ERA for children and is commonly avoided in adults due to its hepatotoxicity.[308] Macitentan is another dual ERA without hepatotoxicity. In contrast, anaemia was observed.[284]

Prostacyclin analogues

Beraprost was the first orally active PGI2 analogue showing improved exercise capacity that persists up to 3–6 months. However, studies did not show haemodynamic or long-term outcome benefits in those patients.[309]

Epoprostenol has a short half-life (3–5 minutes) and is only stable for 8 hours at room temperature. Therefore, Epoprostenol requires cooling and continuous administration. Aprupt interruption of infusion should be avoided to prevent rebound. Epoprostenol additionally improves exercise capacity haemodynamics and reduced mortality in IPAH patients.[310]

Other treatment strategies for IPAH

ECMO- Therapy

The use of va ECMO should be considered for selected patients with PAH and RV failure. A vv ECMO approach is not sufficient, since it does not unload the RV. Va ECMO therapy can be performed as a bridge to recovery and bridge to transplantation.[311-313]

Lung Transplantation for patients with IPAH

LUTX is the last therapeutic options for patients with an inadequate clinical response to medical therapy.[314] Delayed referral combined with organ donor shortage may increase mortality on the waiting list and morbidity at the time of transplantation.[315] The overall 5-year and 10 year survival rate for IPAH patients is at 52–75% and 45–66%.[316, 317] There are two options for IPAH patients: performing heart-lung transplantation or DLUTX. The ISHLT recommends performing DLUTX, due to more experience of the specialized centres.[280]

Balloon atrial septostomy

Ballon atrial septostomy is a palliative treatment option for patients with IPAH. The performance of an inter-atrial right-to-left shunt can decompress the right heart chambers and increase LV preload and cardiac output (CO).[318, 319] This last treatment choice can improve clinical symptoms by improving systemic O_2 transport despite arterial O_2 desaturation.[320]

1.4.3 Chronic thromboembolic pulmonary hypertension

CTEPH occurs in 4 per 1 million adults and in 0.4-9.1% of all patients after acute pulmonary embolism.[321] However, 78,8% and/or 65,1% of all CTEPH patients have a history of pulmonary embolism and/or deep vein thrombosis.[322] Increased levels of Factor VIII and phospholipid antibodies/lupus anticoagulants are associated with CTEPH, however not with other causes of PAH.[250 323, 324] CTEPH is characterized by obstruction or occlusion of subsegmental, segmental or larger pulmonary arteries by postembolic fibrotic material. This condition of precapillary PAH is categorized as Group 4 in the classification of PAH. CTEPH is defined by mPAP >25 mmHg, a PAWP ≤15 mmHg via right heart catheterisation, mismatch on V/Q scintigraphy with at least one large perfusion defect in one segment or in two sub- segments, evidence of pulmonary vascular lesions on computed tomography (CT) and/or magnetic resonance imaging (MRI) or by pulmonary angiography.[286] This findings must last for at least 3 months after effective anticoagulation to differentiate CTEPH from ‘‘subacute’’ pulmonary embolism.[295] Until now, the pathology of CTEPH is not fully understood, however it is thought to be induced by only one or recurrent thromboembolic events followed by vascular wall remodeling in the lungs and subsequent formation of fibrotic vascular occlusions via the interplay of inflammation, infection, inhibition of vascular regeneration, circulating microparticles, abnormal fibrinogen, splenectomy and abnormal circulating phospholipids with reduced thrombus resolution. [325-329] The degree of PAH does not reflect visible thrombotic occlusions.[330]

Treatment of CTEPH

There are three different treatment options available for CTEPH patients. Therapeutic strategies vary according to the anatomical level of obstruction. PAH drug therapies are available for the microvasculature, balloon pulmonary angioplasty (BPA) is indicated for segmental and smaller sub-segmental vessels and PEA is recommended to remove obstructions of the main, lobar, segmental and larger sub-segmental vessels.[331]

Pulmonary endarterectomy

As already discussed above, PEA is the gold standard for CTEPH patients. The 3-year survival rate is known as high with 90% versus 70% in patients who do not undergo surgery.[332] To decide whether patients are operable or not, It is recommended to refer CTEPH patients into specialized high volume centers. High volume centers are defined as centers with a surgical mortality below 5%, a surgical volume of more than 50 PEAs per year and the ability to perform segmental endarterectomy.[333] PEA is performed via median sternotomy requiring CPB enabling gradual cooling to 20°C to ensure deep hypothermic circulatory arrest (DHCA) to improve surgical visualization by keeping the surgical field free of blood from the bronchopulmonary collaterals and thereby guarantee neuroprotection.[235] DHCA is limited to 20 minutes per interval. Usually two periods of DHCA are necessary to perform complete bilateral dissection.[334]

Balloon pulmonary angioplasty

BPA is a percutaneous interventional treatment option for patients with inoperable CTEPH. This procedure involves treatment of vascular CTEPH lesions with semi-compliant balloons at relatively low pressures (about 6–10 atm) in several sessions. BPA was performed for the first time in 2001 and has been improved considerably over the last decades, particularly in Japan.[335, 336]

CTEPH-targeted medical therapy

In CTEPH patients with or without PEA life-long anticoagulation is recommended. Vitamin K antagonists are considered as the therapy of choice due to several decades of clinical experience.[286] Riocigutat is the only approved medical therapeutic options for inoperable or persistent symptomatic CTEPH patients.[286] Until now, it is not recommended to employ medical therapy as a “bridge to surgery”, since it may only delay surgical referral and definitive treatment.[337]

1.4.4 Cystic fibrosis

Cystic fibrosis (CF) is the most prevalent lethal genetic disorder in the western world.[338] In northern Europe and "white" America CF occurs in approximately 1 of 3000 births, whereas only in 1 of 4000–10 000 Latin Americans and 1 of 15000–20000 African Americans.[339, 340] CF was mentioned for the first time in 1938; at that time CF patients died soon after diagnosis.[341] In 2016 a median life expectancy of 47 years was reported in the UK thanks to new emerging treatment options.[342] CF is caused by a mutation in a gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The CFTR protein is expressed in epithelial and blood cells and carries out a pivotal role in chloride channels, inhibits sodium transport through the epithelial sodium channel, regulates the outwardly rectifying chloride channel, ATP channels, intracellular vesicle transport, acidification of intracellular organelles, inhibits endogenous calcium-activated chloride channels and bicarbonate–chloride exchange.[343-345] Until now, already more than 1500 CFTR mutations were found, nevertheless the absence of phenylalanine at position 508 (Phe508del, also known as F508del) accounts for two-thirds of mutated alleles in northern European and North American populations. CF phenotypes have a huge diversity, even in siblings carrying the same CFTR genotype.[345]

CFTR dysfunction leads to lost inhibition of epithelial sodium channels. Therefore, sodium and water were excessively reabsorbed and induce dehydrated airway surfaces. Additional loss of chloride efflux avoids the epithelium from correcting the low water volume on the airway surface, resulting in a decreased lubricating layer between epithelium and mucus. Therefore, cilia were compressed by mucus and therefore inhibit ciliary clearance of mucus. Further, increased mucus on the epithelium are predestined to build plaques and hypoxic niches, which serve as a perfect culture medium for bacterial growth, in particular Pseudomonas aeruginosa.[346-348] The European Union Cystic Fibrosis Diagnostic Working Group and the US Cystic Fibrosis Foundation developed diagnostic algorithms for classic and non-classical cystic fibrosis.[349] These guidelines indicate that the diagnosis of cystic fibrosis consists out of specific clinical characteristics, in combination with biochemical and/or genetic factors of CFTR dysfunction. Cystic fibrosis can be diagnosed according to specific clinical features in conjunction with increased sweat chloride concentrations (30–59 mmol/L for infants < 6 months, 40–59 mmol/L for children and > 60 mmol/L for adults;), and two disease-causing CFTR mutations.[350] Newborn screening has to be performed by measuring the immunoreactive trypsinogen (IRT) in blood spots drawn from newborn infants. IRT testing is not specific for CF, however increased IRT concentrations suggest pancreatic injury. Infants with high IRT

concentrations require further assessments: either another IRT test in 1–3 weeks or a genetic analysis of the initial blood spot for a specified group of CFTR mutations.[351, 352] Positive screening results only indicate that the child has an increased risk for CF; a sweat test still remains outstanding to confirm the diagnosis.[353]

Conservative treatment options for CF

Currently there is no universal treatment strategy available which is applicable for each individual diagnosed with CF. CF therapy requires a multidisciplinary, patient-centred approach in specialized CF centres. Existing therapeutic options for CF related respiratory diseases can be sub-divided into three categories comprising clearance of airway mucus, airway infection, and optimisation of nutritional status.[354]

Airway mucus clearance and reduction in airflow obstruction

The bioengineered nebuliser Dornase alfa, a recombinant human deoxyribonuclease (rhDNase), acts by breaking up extracellular DNA released from neutrophils within sputum, making sputum thinner and easier to expectorate. [355, 356] Nebulized hypertonic (7%) saline and inhaled dry-powder mannitol create an osmotic gradient to pull water into the airway lumen, thus hydrating the mucus and ameliorating mucociliary clearance.[357]

Airway anti-inflammatory agents

Corticosteroids have been used to reduce acute airway inflammation and airflow obstruction in CF patients. Two Cochrane reviews found reduced disease progression after short-term use, but insufficient evidence supporting long-term corticosteroid therapy due to harmful systemic side effects.[358, 359] Immunomodulatory/ anti-inflammatory effects of *azithromycin* have been reported; the exact mechanism remains unknown. Clinical studies reported increased lung function and a reduction of pulmonary exacerbations after azithromycin intake.[360, 361]

Antibiotic therapy

Pulmonary exacerbations are commonly treated with short courses of antibiotic intake; either orally for mild exacerbations or intravenously for more severe exacerbations. Topical application is performed for maintenance antibiotic therapy to suppress bacterial count and avoid chronic airway infections of *Pseudomonas aeruginosa.* For CF patients

colistimethate sodium (colistin), tobramycin and aztreonam are commonly prescribed. [362, 363]

Lung transplantation for CF

LUTX is the last therapeutic option for CF patients. In 1983 the first combined heart-lung transplantation was performed for a CF patient.[364] In 1989 the first DLUTX for CF was performed in Marseille.[365] In contrast to other end stage lung diseases, candidates for LUTX with CF are of a younger age group. In the Unites States 25 children out of 200 patients, undergo LUTX each year. However, LUTX is not a cure for patients diagnosed with CF; the median survival rate for adults after LUTX is 6.4 years.[366] Therefore, it is important to consider two important issues for listing CF patients at the adequate time-point: On one hand listing must be performed early enough for the CF patient to survive the length of time needed to wait for an organ, on the other hand the procedure must provide advantage in terms of survival.[366, 367]

1.4.5 Idiopathic pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a specific type of *idiopathic interstitial pneumonia*.[368] The incidence of IPF is estimated to range between 2.8 and 18 cases per 100 000 people per year.[369, 370] It is known as a severe chronic inflammatory lung disease with progressive interstitial fibrosis with a high mortality and morbidity rate. The median survival time is only 2–3 years after diagnosis; as therapeutic options remain limited.[371] IPF appears through repetitive local micro-injuries in the alveolar epithelium as a consequence of multiple interacting genetic and environmental risk factors, such as a history of cigarette smoking, exposure to metal and wood dust, agriculture, viruses, stone and silica.[372, 373] Micro-injuries initiate aberrant epithelial-fibroblast communication and induce matrix-producing myofibroblasts. Consequently, leading to accumulation of extracellular matrix and remodeling of lung interstitum.[374] IPF is diagnosed on the basis of specific radiological or histological findings for usual interstitial pneumonia UIP after exclusion of alternative causes. Typical patterns in high resolution CT scans of the chest show a predominantly bilateral, peripheral and basal distribution of reticular opacities wit traction bronchiectasis and subpleural cystic airspaces with honeycombing (diameter: 3-10mm).[371] Patients presenting basal and subpleural abnormalities without honeycombing are considered to have a possible UIP. In these patients surgical lung biopsy has to be performed. Histopathological findings consist out of areas of fibrosis, architectural deformation, microscopic *honeycombing* and aggregates of proliferating fibroblasts and myofibroblasts, called fibroblast foci within a myoxid appearing matrix that is typically found between fibrotic and normal lung tissues. Fibroblast foci are known as a key histopathological finding for UIP by representing active disease and their absence is an exclusion criterion for definitive UIP.[375]

Conservative treatment options for IPF

Delayed referral of IPF patients to specialized centers is associated with increased mortality. Therefore, it is of great importance to send patients with established or suspected diagnosis for IPF immediately to specialized clinics and initiate disease-modifying therapies, non-pharmological support or even list patients for DLUTX as soon as possible.[376]

Antifibrotic therapy

Until now, two antifibrotic therapies, nintedanib and pirfenidone exist. Both drug therapies have shown to moderately slow down disease progression. Nintedanib (tyrosine kinase inhibitor) reduces lung function decline and the incidence of acute exacerbations. It inhibits fibrostic pathogenesis by supressing multiple involved signalling receptors such as the fibroblast growth factor receptor, platelet –derived growth factor receptor and the vascular endothelias growth factor receptor.[377] Pirifenidone (pyridine) combines anti-inflammatory, antioxidant, and antifibrotic actions. It's known to regulate TGF-B in vitro, and inhibits fibroblast and collagen synthesis in an animal model. Pirifenidone shows similar therapeutic effects to Nintedanib. Both therapeutic options do not show survival benefits. Adverse effects for both therapeutics include increased transaminases and gastrointestinal and dermatological symptoms.[378]

Lung Transplantation for patients with IPF

Until now, LUTX remains the only therapeutic strategy for patients with IPF. DLUTX can improve the quality of life and survival, with a median survival rate of 4.7 years.[379, 380] Single and bilateral LUTX can be performed.[381]

1.5 Aims and Hypotheses

This prospective, single center research work aimed to study the qualitative and quantitative perioperative cytokine “storm” during different major thoracic procedures with or without extracorporeal supporting systems comprising LUTX on ECMO, PEA on CPB and lobectomy without ECC. Patients with end-stage pulmonary disease undergoing LUTX such as COPD, CF, IPAH and IPF were further investigated. The perioperative inflammatory response during LUTX, PEA or lobectomy was observed in serum cytokine concentrations and clinically by performing SOFA. Our objective was to find predisposing *clinical* (SOFA) and *experimental* (cytokines) risk factors to detect adverse clinical outcome after extensive thoracic procedures employing ECC.

1.5.1 Hypothesis No. 1:

Null hypothesis H0: The type of thoracic surgical procedure has no influence on the quantitative or qualitative inflammatory/immune response.

Alternative hypothesis H1: The type of thoracic surgical procedure has an impact on the quantitative or qualitative inflammatory/immune response.

1.5.2 Hypothesis No. 2:

Null hypothesis H0: Different underlying diagnoses of patients undergoing DLUTX have no influence on the quantitative or qualitative inflammatory/immune response.

Alternative hypothesis H1: Different underlying diagnoses of patients undergoing DLUTX have an influence on the quantitative or qualitative inflammatory/immune response.

CHAPTER TWO: RESULTS

2.1 Prologue

Synthetic, non-degradable membranes induce an acute inflammatory and anti-inflammatory immune response. In the following prospective, observational research work we determined the acute cytokine response in patients undergoing extensive thoracic surgery on ECC, including patients with end-stage respiratory disease undergoing DLUTX on ECMO, CTEPH patients undergoing PEA and lung cancer patients undergoing Lobectomy without ECC. Cytokines including IL-6, IL-10, sST2 and TNF-alpha were measured before, after surgery, and during the first 5 postoperative days. Clinically we determined inflammation via the SOFA score once a day during the ICU stay. Additionally, we performed outcome analysis including surgical revision, 30d mortality and ICU complications such as re-intubation and hemofiltration.

In the following pages, a copy of the original publication, published in the annals of translational medicine (open source) is given. Author contributions are delineated on page II.

Original Article

Transient perioperative inflammation following lung transplantation and major thoracic surgery with elective extracorporeal support: a prospective observational study

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Background: The clinical relevance of inflammation induced by elective perioperative extracorporeal membrane oxygenation (ECMO) usage as an integral part of modern lung transplantation (LUTX) remains elusive. The aim of this study was to determine the perioperative cytokine response accompanying major thoracic surgery employing different extracorporeal devices comprising ECMO, cardiopulmonary bypass (CPB), or no extracorporeal circulation in relation to inflammation, clinically tangible as increased sequential organ failure assessment (SOFA) score, called SOFA.

Methods: In this prospective, observational pilot study 42 consecutive patients with end-stage pulmonary disease undergoing LUTX; 15 patients with chronic thromboembolic pulmonary hypertension (CTEPH) undergoing pulmonary endarterectomy and 15 patients with lung cancer undergoing major lung resections were analysed. Cytokine serum concentrations and SOFA were determined before, at end of surgery and in the following postoperative days.

Results: LUTX on ECMO and pulmonary endarterectomy (PEA) on CPB triggered an immediate increase in cytokine serum concentrations at end of surgery: IL-6: 66-fold and 71-fold, IL-10: 3-fold and 2.5-fold, ST2/IL-33R: 5-fold and 4-fold and SOFA: 10.5±2.8 and 10.7±1.7, that decreased sharply to baseline levels from postoperative day 1–5. Despite low perioperative mortality (3 patients, 4.1%) extremely high SOFA ≥13 was associated with mortality after LUTX. Delta-SOFA distinguished survivors from non-survivors: −4.5±3.2 *vs.* −0.3±1.5 (P=0.001). Increased IL-6 serum concentrations were predictive for increased SOFA (sensitivity: 97%, specificity: 80%). Peak cytokine serum concentrations correlated with ECC duration, maximal lactate, transfusion of red-blood-cells, fresh-frozen-plasma, and catecholamine support.

Conclusions: LUTX and PEA on extracorporeal circulation with an excellent outcome triggered an immediate rise and concomitant fall of inflammation as observed in cytokine serum concentrations and SOFA. High absolute SOFA in the presence of an uncomplicated postoperative course may pertain to specific management strategies rather than organ failure.

Keywords: Extracorporeal membrane oxygenation (ECMO); lung transplantation (LUTX); pulmonary endarterectomy (PEA); perioperative inflammation; sequential organ failure assessment (SOFA)

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Introduction

Over the years, the indications for deployment of extracorporeal membrane oxygenation (ECMO) have expanded from resuscitation of patients with acute respiratory and cardiac failure to elective and semi-elective thoracic surgical procedures. ECMO is a technology capable of providing short- and long-term mechanical support to the heart, lungs, or both (1). At our institution the first case series report on the successful use of ECMO for the treatment of graft failure after cardiac transplantation—when weaning from cardiopulmonary bypass(CPB) was not possible—was published in 2001 (2). In the same decade, ECMO gradually evolved as a safe intraoperative support alternative to CPB, enabling the resection of locally advanced intrathoracic malignancies and complex tracheobronchial procedures (3). Subsequently, the use of veno-arterial (v/a) ECMO support was integrated in standard surgical treatment strategies for patients undergoing lung transplantation (LUTX) and for postoperative hemodynamic and respiratory stability in selected patients with poor initial graft function (prolonged ECMO). Veno-venous (v/v) ECMO is increasingly employed to bridge patients with respiratory failure to LUTX (4-6).

Previous investigations have shown that the use of CPB induces a brief pro-inflammatory response, clinically determined by the sequential organ failure assessment (SOFA) followed by a long-lasting second phase of immune suppression (7). The SOFA score was designed to clinically measure organ dysfunction by assessing the respiratory, coagulation, hepatic, cardiovascular, renal and neurological function in patients admitted to the ICU (8). The exposure of a patient's blood to foreign surfaces of the CPB circuit is known to imbalance the inflammatory system via blood flow shear stress, expression of cytokines, activation of the complement system and dysfunctions of the coagulation system (1,9). This broad wave of systemic inflammation has been linked to adverse clinical outcomes ranging from mild adverse effects such as fever or diffuse tissue edema, to moderate adverse effects comprising pathological hemodynamic instability or coagulopathy, to severe complications including acute organ injury and even mortality (10,11).

A similar response induced by ECMO was observed in patients with acute refractory cardiac and respiratory failure requiring immediate life-saving circulatory and respiratory support (12). Experimental and animal studies have shown that already two hours after initiation of ECMO support, pro- and anti-inflammatory cytokines [interleukin (IL)-1β, IL-6, IL-10 and tumour necrosis factor (TNF)-α] were significantly up-regulated (13,14). Several studies investigated the inflammatory response to ECMO during resuscitation, whereas the use of elective intraoperative and prolonged ECMO support has never been systematically investigated (15).

The main objective of this study with observational design was to investigate the perioperative inflammatory response of thoracic surgical procedures employing extracorporeal circulation (ECC) comprising LUTX on ECMO, pulmonary endarterectomy (PEA) on CPB; and patients undergoing pulmonary resections without ECC. Sub-analysis was performed among LUTX patients with different underlying diagnoses [chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), idiopathic pulmonary fibrosis (IPF) and idiopathic pulmonary arterial hypertension (IPAH)]. The perioperative inflammatory response was elicited by cytokine measurements and SOFA. We attempted to identify predisposing factors of clinical(SOFA) and experimental origin(cytokines) for the early detection of worse clinical outcome after major thoracic surgery, employing elective ECMO or CPB. We present the following article in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/atm-20-4771).

Methods

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Institutional Ethics Committee of the Medical University of Vienna (EK1363/2018). Written informed consent was obtained from all study participants.

Study design, setting and patients

The study was designed as an explorative prospective pilot and cohort study combining clinical and experimental

research. It was designed as a purely observational study and performed at the Medical University of Vienna.

We included 42 consecutive patients with end-stage pulmonary disease [COPD (n=15), CF (n=15), 123 IPF (n=7), IPAH (n=5)] undergoing LUTX on ECMO; 15 consecutive chronic thromboembolic pulmonary hypertension (CTEPH) patients undergoing PEA on CPB and 15 consecutive lung cancer patients undergoing major lung resections without ECC during a period of 12 months, from May 2018 until April 2019. We excluded pregnant women, patients who were younger than 18 years and patients who did not give written informed consent. Blood samples were drawn before surgery, at ICU admission, once on each of the 3 following post- operative days (PODs) and at POD5. IL-6, IL-10, ST2/IL-33R, TNF-α and transforming growth factor (TGF)-β serum concentrations were measured by commercially available enzyme-linked immunosorbent assay (ELISA) kits.

Sandwich ELISA technique

IL-6, IL-10, ST2/IL-33R, TNF-α and Transforming Growth Factor (TGF)-β serum concentrations were measured by commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, Minnesota, United 130States) according to the manufacturers' instructions. 96-well microplates were incubated with capture antibodies (Mouse anti-human IL-6, IL-10, ST2, TNF-α and TGF-β) overnight at room temperature. Blocking was done with assay buffer. After incubation with serum samples and washing, HRP-conjugated detection antibodies were added (Biotinylated goat anti-human IL-6, IL-10, ST2, TNF-α and TGF-β). A color reaction was obtained with peroxidase reagent tetramethylbenzidine (TMB) (Sigma-Aldrich Corp., St. Louis, MO, USA) and the optical density (OD) was read at 450 nm using an absorbance microplate reader for ELISA, the Infinite F50 (Tecan, Männedorf, Switzerland).

Perioperative management

Anesthesia

All patients enrolled received standard perioperative monitoring after entering the operating room. Anesthesia was induced with 2 mg Midazolam, Fentanyl 0.2 µg/kg, Propofol 2 mg/kg and Cis-Atracurium 0.2 mg/kg (PEA and LUTX)/Rocuronium 0.5 mg (lung resection). Antibiotic prophylaxis was administered: piperacillin/tazobactam 4.5 g for PEA and LUTX and cefuroxime for lung resection within 30 to 60 minutes before incision. A Swan-Ganz catheter and a central venous catheter were placed into the right or left jugular vein to measure pulmonary artery pressures in patients undergoing LUTX and PEA. Anesthesia was maintained with fentanyl via perfusion or bolus and propofol 6 mg/kg/h for PEA and LUTX and sevoflurane 1 minimal alveolar concentration for lung resection. Transesophageal echocardiography was installed in patients undergoing PEA or LUTX to monitor cardiac function, volume status and adrenergic support during surgery. While an initial dose of 60 IU/kg of heparin was administered before initiation of intraoperative veno-arterial (v/a) ECMO for patients undergoing LUTX, 400 IU/kg of heparin were given before introduction of CPB for patients undergoing PEA. Normothermic conditions of 37 ℃ were pursued for patients undergoing LUTX and lung resection. Patients undergoing PEA were cooled to 18 ℃ (deep hypothermia) to endure periods of complete circulatory arrest.

Surgery

Donor lungs were harvested during multi-organ procurement preserved with colloid containing low potassium solution and kept inflated during transport. LUTX was performed through bilateral thoracotomy or clamshell incision. LUTX was performed with intraoperative central v/a ECMO with and without prolongation into the postoperative period. In patients with prolonged v/a ECMO, central cannulation was switched to the peripheral location in the groin after implantation of the lungs (16).

PEA was performed through a median sternotomy requiring CPB with bicaval cannulation. After aortic cross-clamping, cardioplegia was administered and deep hypothermic circulatory arrest was employed to improve visualization of the pulmonary arteries. Bilateral PEA was performed sequentially in all patients (17).

Lung resections were performed via muscle-sparing antero-lateral thoracotomies. Only patients undergoing anatomic pulmonary resection (lobectomy/pneumonectomy) during single lung ventilation (double-lumen intubation) were included in this study.

Extracorporeal circulation (ECC)

Bridging strategies were chosen according to patient's hemodynamic and respiratory conditions. Patients with severe hypoxic respiratory failure received a v/v ECMO (Cardiohelp, Oygenator Quadrox)/(Xenios, Oxygenator Hilite) with a 2-site (femoro-jugular) or single-site large-

bore double-lumen cannula ranging from 27F to 31F (Avalon Laboratories, Los Angeles, Calif) (18).

All patients were transplanted on intraoperative heparin-bound v/a ECMO (Medtronic Carmeda), hollow-fiber oxygenator (Medtronic), centrifugal pump (Biomedicus), flow probes and 3/8- inch internal diameter (18).

PEA for CTEPH patients was performed with CPB. The CPB circuit was primed (1,000 mL crystalloid and 500 mL colloid solution together with 5,000 IE heparin, and 100 mL mannitol 20%) according to institutional standards. CPB was performed using non-pulsatile flow at 2.5 L/min/m^2, a non-heparin-coated circuit, and a membrane oxygenator (Quadrox™, Maquet, Hirrlingen, Germany, or Capiox, Terumo, Eschborn, Germany) (19).

For prolonged ECMO support a femoro-femoral v/a ECMO using 17F to 19F drainage and 15F to 17F reperfusion cannulas was inserted (all Bio-Medicus Cannula, Medtronic Inc, St Paul, Minneapolis). Anticoagulation during prolonged ECMO support was managed by subcutaneous administration of low-molecular-weight heparin. ECMO was continued until patients were hemodynamically stable, had normal chest X-ray, adequate oxygenation (fraction of inspired oxygen <0.5), low ventilation pattern and had a normalized fluid balance.

Postoperative management

Patients undergoing LUTX received induction therapy with alemtuzumab in 84% or Anti-thymocyte globulin in 3% or no induction therapy (13%) directly after ICU admission. Maintenance therapy was employed in all patients with tacrolimus, mycophenolat-mofetil (only in the non-alemtuzumab group) and corticosteroids. All patients undergoing LUTX received postoperative anti-infectious prophylaxis therapy with piperazillin/tazobactam, a lifelong Pneumocystis prophylaxis with trimethoprim-sulfamethoxazole, prophylactic inhalation therapy with amphotericin B and gentamicin and CMV prophylaxis including CMV hyperimmuno-globulines (POD 1, 7, 14 and 21) together with valganciclovir. Patients undergoing PEA received piperazillin/tazobactam or cefazolin for postoperative antibiotic prophylaxis. Patients undergoing lung resection did not routinely receive postoperative antibiotic therapy.

Definition of SOFA

SOFA was assessed every 24 h for all patients admitted to the ICU (LUTX and PEA patients). The SOFA score was calculated by assessing each of the 5 organ dysfunctions (respiratory, coagulation, hepatic, cardiovascular and renal) from 0–4 points. The Assessment of the neurological function by using the Glasgow coma scale (GCS) was excluded due to limited evaluability in actively sedated patients. The following data was collected: need for mechanical ventilation, arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) ratio, platelet count, bilirubin, mean arterial pressure, doses of adrenergic agents, creatinine, and urine output (7).

Statistical analysis

Normally distributed data were reported as mean ± SD, non-normal distributions as median (range). Mann-Whitney U test was employed for non-normally distributed data and *t*-tests for parametric data. Pearson's correlation-coefficient r was employed to measure the strength of the linear relationship between cytokine concentrations and clinical data. We plotted the receiver operating characteristic (ROC) curve, calculated the Youden Index to identify optimal cut-offs for cytokine concentrations at the end of surgery. We performed binary logistic regression to evaluate the negative predictive value (NPV), positive predictive value (PPV), sensitivity, specificity, and the OR to predict postoperative SOFA (>10 points). Chi-square tests were employed to test relationships between categorical variables. Kaplan Mayer analysis was performed for survival analysis. The level of statistical significance was set at 0.05 (two-tailed P values). Statistical analyses and visualization were performed using SPSS software (version 25; IBM SPSS Inc., IL, USA) and GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA). Boxplots were designed as followed: Box: 1st to 3rd quartile, Bar: median, Whiskers: percentile 5-95, Outliers: all shown as dots.

Results

Demographic data and perioperative characteristics are depicted in *Table 1*. Five patients (11%) underwent Re-LUTX due to chronic lung allograft dysfunction. Among these patients three were initially diagnosed with CF, one had IPF and one was initially suffering from IPAH.

Nine percent of all patients undergoing LUTX (COPD, CF, IPF, and IPAH) were bridged to transplantation via semi-elective v/v ECMO, 100% received intraoperative, elective v/a ECMO and 19% received prolonged semi-elective v/a ECMO after surgery, respectively. All patients

Table 1 Basic demographic, procedural and outcome data

Diagnosis	COPD	CF	IPF	IPAH	CTEPH	Lung cancer
Surgery	LUTX	LUTX	LUTX	LUTX	PEA	Lung resection
Basic demographic data						
Number [%]	15 [100]	15 [100]	7 [100]	5 [100]	15 [100]	15 [100]
Age (years) mean ± SD	59±6	29±8	51±13	39±10	59±15	69±7
Female:male ratio, n [%]	4 [27]:11 [73]	9 [60]:6 [40]	2 [29]:5 [71]	3 [60]:2 [40]	6 [40]:9 [60]	8 [53]:7 [47]
Primary surgery, n [%]	15 [100]	13 [87]	4 [58]	4 [80]	15 [100]	15 [100]
Retransplantation, n [%]	–	2 [13]	3 [42]	1 [20]	–	–
Extracorporeal support, n [%]						
ECMO preoperative	–	2 [13]	2 [28]	–	–	–
CPB	–	–	–	–	15 [100]	–
Intraoperative ECMO	15 [100]	15 [100]	7 [100]	5 [100]	–	
ECMO postoperative	2 [13]	2 [13]	3 [42]	1 [20]	2 [13]	–
Intraoperative characteristics						
Time (min)						
Length of ECC mean ± SD	183±31	200±48	204±91	218±51	270±64	–
Length of surgery mean ± SD	315±70	324±71	384±89	341±96	465±136	146±59
Vasoactive administration						
Noradrenaline, n [%]						
<0.1 µg/kg/min	11 [73]	8 [53]	1 [14]	3 [60]	7 [47]	15 [100]
>0.1–0.5 µg/kg/min	4 [27]	7 [46]	6 [86]	–	7 [47]	–
>0.5 µg/kg/min	–	–	–	2 [40]	1 [6]	–
Dobutamine, n [%]	0 [0]	0 [0]	0 [0]	0 [0]	1 [7]	0 [0]
Blood and coagulation products						
PRBCs mean ± SD	4.6±3	8.6±8	10±9	11±11	6±8	1±3
FFPs mean ± SD	11±5	14±11	15 ±15	18 ±17	4±3	0
Fibrinogen, n [%]						
0 g	10 [67]	9 [60]	2 [28.5]	2 [40]	4 [26]	14 [93]
0–2 g	5 [33]	3 [20]	2 [28.5]	–	7 [47]	–
3–5 g	–	3 [20]	1 [14.5]	2 [40]	3 [20]	–
>5 g	–	–	2 [28.5]	1 [20]	1 [7]	1 [7]
Tranexamic acid, n [%]						
0 mg	14 [93]	12 [80]	4 [57]	1 [20]	1 [7]	15 [100]
500 mg	–	1 [7]	2 [28]	2 [40]	3 [20]	–
1,000 mg	1 [7]	2 [13]	1 [14]	2 [40]	11 [73]	–
Immunosuppression, n [%]						

Table 1 (*continued*)

Table 1 (*continued*)

Diagnosis	COPD	CF	IPF	IPAH	CTEPH	Lung cancer
Methylprednisolone 1 g	15 [100]	15 [100]	7[100]	5 [100]	–	–
Hydrocortisone 100 mg	–	–	–	–	15 [100]	–
Measurement of serum parameters						
Max. BL (mg/dL) mean ± SD	3.1±1.2	3.5±1.1	4.2±1.4	4.0±1.8	4.3±0.8	1.5±0.7
Min. HB (pg/dL) mean ± SD	9.1±0.9	9.3±1.4	9.6±1.2	9.0±1.4	9.2±0.9	10.4±1.8
Postoperative characteristics (first 24 h)						
SOFA-Score mean ± SD	9.2±1.4	10.9±3	11.2±4.6	11.8±1.3	10.72±1.7	–
qSOFA-Score mean ± SD	–	–	–	–	–	0 [0]
SBP <100	–	–	–	–	–	0 [0]
RR >22	–	–	–	–	–	0 [0]
GCS <15	–	–	–	–	–	0 [0]
Outcome analysis, n [%]						
Revision, mean ± SD	1 [7]	0 [0]	0 [0]	0 [0]	0 [0]	2 [13]
VAC, mean ± SD	1 [7]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]
POS, mean ± SD	2 [13]	0 [0]	2 [25]	2 [40]	2 [13]	–
30-d mortality, mean ± SD	1 [7]	0 [0]	1 [12]	0 [0]	1 [7]	0 [0]
HF, mean ± SD	1 [7]	1 [7]	1 [7]	2 [40]	2 [13]	0 [0]

BL, blood lactate concentration; bpm, beats per minute; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; CPB, cardiopulmonary bypass; d, day; ECC, extracorporeal circulation; ECMO, extracorporeal membrane oxygenation; FFP, fresh frozen plasma; HB, haemoglobin; G/l, Giga per liter; HR heart rate; IPAH, idiopathic pulmonary hypertension IPF idiopathic pulmonary fibrosis; LUTX, lung transplantation; n, number; PEA, pulmonary endarterectomy; POS, psycho-organic syndrome; PRBCs, packed red blood cells; RR, respiratory rate; SBP, systolic blood pressure; SD, standard deviation; SOFA, sequential organ failure assessment score; VAC, vacuum assisted closure-therapy; WBC, white blood cells.

undergoing PEA (CTEPH) had CPB during surgery and 13% required postoperatively prolonged semi-elective v/a ECMO support. Patients undergoing lung resection for cancer did not require ECC support. The peak SOFA was at 10.5±2.8 for LUTX on ECMO and 10.7±1.7 for PEA on CPB patients at end of surgery and decreased steadily thereafter.

Treatment differences between study groups

In patients undergoing LUTX on ECMO support there was no difference among the different underlying diagnoses (COPD, CF, IPF, and IPAH) with respect to the number of administered units of packed red blood cells (PRBC) (P=0.119), units of fresh frozen plasma (FFP) (P=0.623), grams of fibrinogen (P=0.083), dosage of noradrenalin (P=0.107), blood lactate concentration (P=0.311), haemoglobin concentration (P=0.841), ECC-time (P=0.526) and time of surgery (P=0.187). In contrast there are inherent treatment differences comparing patients undergoing LUTX on ECMO and patients undergoing PEA supported by CPB: units of PRBC (P=0.026), units of FFP (P<0.001), blood lactate concentration (P=0.021), ECC time (P<0.001) and time of surgery (P<0.001), but no difference in the administration of grams of fibrinogen (P=0.156) and dosage of noradrenalin (P=0.940, *Table 1*).

Increased postoperative cytokine release

Baseline preoperative cytokine concentrations did not reveal statistically significant differences between patients planned for LUTX on ECMO, PEA on CPB or pulmonary

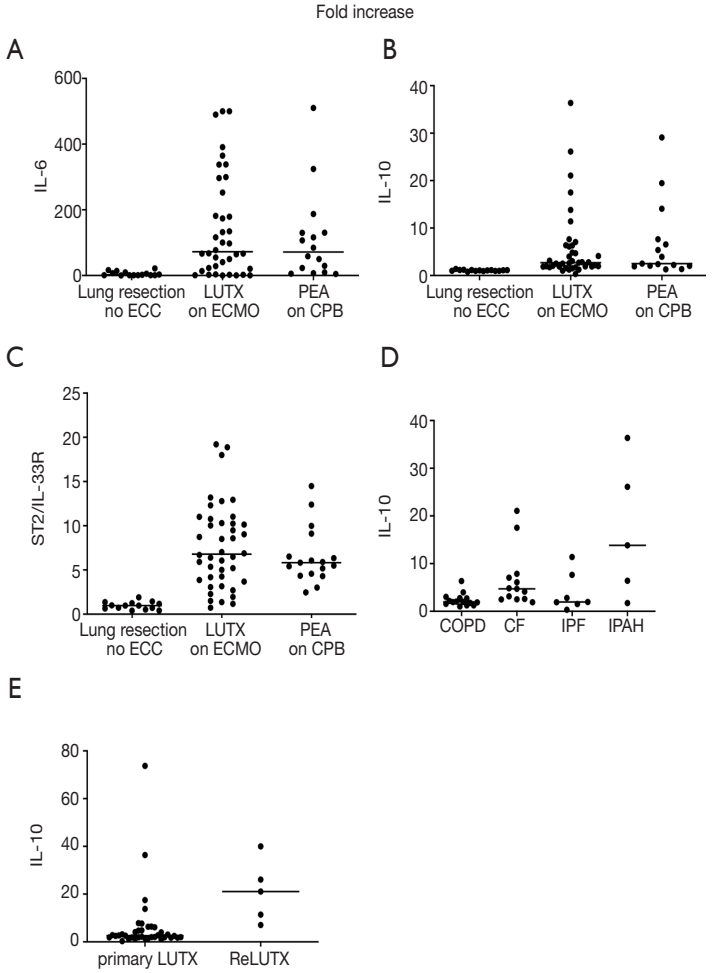

Figure 1 Increased cytokine expression after LUTX and PEA were depicted as fold increase (increase from baseline prior to surgery to ICU admission). Serum cytokine expressions including IL-6 (A), IL-10 (B) and ST2/IL33 (C) of patients undergoing LUTX with ECMO and PEA with CPB; and patients undergoing lung resection without ECC were depicted. A statistically significant increase in IL- 10 serum concentrations among patients undergoing LUTX with CF and IPAH compared to COPD patients from baseline prior to surgery to peak concentrations after surgery were shown in (D). Significantly increased IL-10 serum concentrations from baseline to end of surgery in patients undergoing primary LUTX compared to patients undergoing Re-LUTX are presented in (E). LUTX, lung transplantation; PEA, pulmonary endarterectomy; ECMO, extracorporeal membrane oxygenation; ECC, extracorporeal circulation; COPD, chronic obstructive pulmonary disease; LUTX, lung transplantation.

resections without extracorporeal support.

IL-6 serum concentrations increased 66-fold after LUTX on ECMO support, 71-fold after PEA on CPB and 2-fold in patients undergoing lung resection without ECC (*Figure 1A*).

Patients undergoing LUTX on ECMO had an 8-fold, PEA on CPB a 7-fold and patients undergoing pulmonary resections without ECC had no increase of IL-10 serum concentrations, respectively (*Figure 1B*).

ST2/IL-33R serum concentrations increased 5-fold in patients who underwent LUTX on ECMO, 4-fold in patients after PEA on CPB, but not in patients with pulmonary resections without ECC (*Figure 1C*).

TNF-α serum concentrations increased 15-fold in LUTX on ECMO, 3-fold in PEA on CPB but not in pulmonary resections without ECC. There were no alterations in TGF-β serum concentrations.

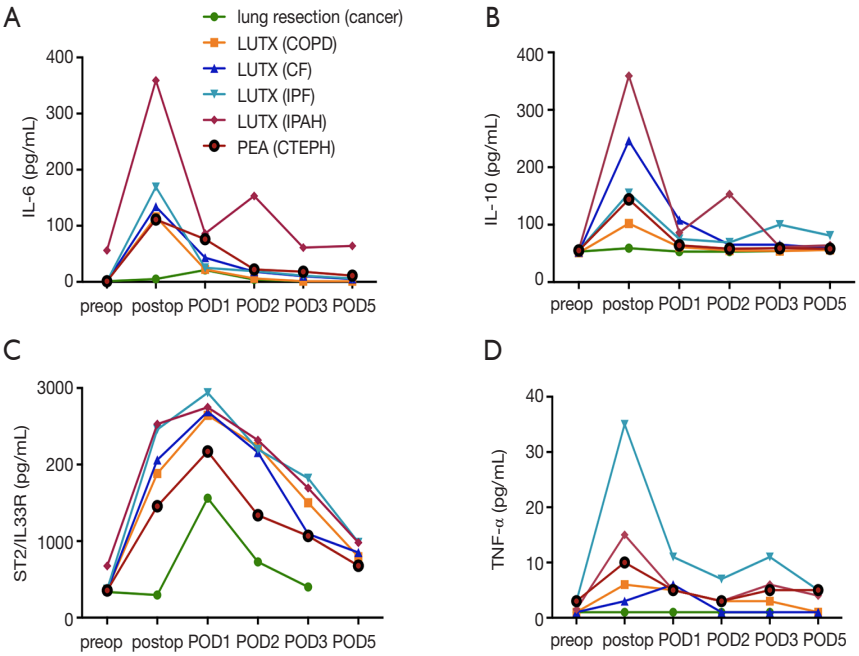

Figure 2 Dynamic cytokine-expression in the perioperative period. The dynamic perioperative cytokine release is shown in IL-6 (A), IL-10 (B), ST2/IL33 (C) and TNF-α (D).

The underlying diagnoses influence IL-10 serum concentrations in patients undergoing LUTX (COPD, CF, IPF, IPAH)

IL-10 serum concentrations increased significantly from baseline prior to surgery to peak concentrations after surgery between patients undergoing LUTX for COPD [51 (range, 49–69) to 102 (range, 62–321) pg/mL] and CF [52 (range, 48–88) to 246 (range, 125–6,514) pg/mL] (P<0.001) or IPAH [56 (range, 50–76) to 705 (range, 100–2,104) pg/mL] (P<0.019) (*Figure 1D*).

Increased preoperative IL-10 concentrations in patients bridged to LUTX

Only preoperative IL-10 concentrations were significantly increased in patients with implanted v/v ECMO: [55 (range, 54–908) pg/mL] compared to no ECMO bridging: [53 (range, 42–104) pg/mL] (P=0.045, *Figure 1E*).

Dynamic changes of cytokine-expression after surgery

IL-6 serum concentrations increased significantly from baseline to end of surgery and decreased significantly at POD1 in all patients. From POD1 to POD2 there was a significant decrease in IL-6 concentrations in patients with LUTX (COPD, CF) and PEA (CTEPH) and a second significant increase in patients with LUTX (IPAH) (*Figure 2A*). IL-6 serum concentrations increased 116-fold in COPD, 28-fold in CF, 31-fold in IPF, 36-fold in IPAH, 71-fold in CTEPH and 2-fold in patients with lung resection from baseline to end of surgery.

IL-10 serum concentrations increased in LUTX (COPD, CF, IPAH) and PEA (CTEPH) patients form baseline to end of surgery; and decreased statistically significant in the following postoperative days (*Figure 2B*). IL-10 serum concentrations increased 2-fold in COPD, 5-fold in CF, 2-fold in IPF, 13-fold in IPAH, 2.5-fold in CTEPH and 1-fold in patients with lung resection from baseline to end of surgery.

Peak ST2/IL-33R concentrations were detected at POD1 in all study groups followed by a decrease starting on POD2. ST2/IL-33R levels rose significantly in patients with LUTX (COPD, CF) and PEA (CTEPH) from baseline to end of surgery, from end of surgery to POD1 and decreased significantly at all consecutive time points. Patients with LUTX (IPAH) had only a significant increase from baseline to end of surgery and a significant decrease

from POD2 to POD3 and POD5. The lung resection group rose significantly from end of surgery to POD1 and decreased at all following time-points (*Figure 2C*). ST2/IL-33R serum concentrations increased 4-fold in COPD, 5-fold in CF, 5-fold in IPF, 3-fold in IPAH, 4-fold in CTEPH and 1-fold in patients with lung resection from baseline to end of surgery.

TNF-α serum concentrations rose significantly in LUTX (COPD, IPF) and PEA (CTEPH) patients from baseline to end of surgery. A significant decrease from end of surgery to POD1 was only observed in patients with PEA (CTEPH) (*Figure 2D*). TNF-α serum concentrations increased 6-fold in COPD, 1-fold in CF, 7-fold in IPF, 3-fold in IPAH, 2.5-fold in CTEPH and 1-fold in patients with lung resection from baseline to end of surgery.

There were no alterations in TGF-β serum concentrations in all time-points.

Correlation between cytokine expression and clinical parameters

Correlations among clinical parameters and fold-increase of cytokine concentrations from baseline prior to surgery to postoperative peak serum concentrations are depicted in *Table 2*. A significant correlation among IL-10, ST2/IL33R, CRP serum concentrations and ECC length could be observed. Total operation time did not show any impact on serum cytokine concentrations. High maximum lactate concentrations were significantly associated with high fold-increase of IL-10, TGF-β and CRP levels. Packed red cells counts, fresh frozen plasmas (FFPs) and dosages of noradrenaline correlated significantly with IL-10 and IL-6 fold-increases. Low haemoglobin levels were only associated with statistically significant IL-6 fold-increases.

There was no association between cytokine serum concentrations and primary graft dysfunction (PGD) grading (supplement).

Cytokines are prognostic of inflammation as defined by SOFA

The sensitivity, specificity, PPV, NPV and OR of all cytokines for predicting increased perioperative inflammation as defined by SOFA criteria are detailed in *Table 3*. The following Youden indices divided patients into groups of low and high serum concentrations of the respective cytokines for SOFA >10: IL-10 311 ng/mL, IL-6 69.0 ng/mL, TNF-α 2.3 ng/mL, ST2/IL33R 2,159 ng/mL, TGF-β 17.2 ng/mL. IL-6 serum concentrations at end of surgery had the highest OR 18.6 and the highest sensitivity of 97% for SOFA, respectively.

SOFA and relative SOFA changes (Δ-SOFA) are associated with postoperative outcome

Kaplan Mayer survival analysis (cut-off SOFA ≥13) revealed a significantly greater survival for patients with SOFA <13 (P=0.02).

Focusing on patients who underwent LUTX a change in SOFA score (Δ-SOFA) from end of surgery to POD3 was at −3.9±3.3 for COPD, −5.5±2.5 for CF, −3±4.9 for IPF, and −5.2±2.3 for IPAH. Patients who underwent PEA for CTEPH displayed a Δ-SOFA of −4.7±3.5 (*Figure 3A*). Δ-SOFA decreased at −1.5±4.9 for re-intubated and −4.6±3.3 for patients who remained extubated (P<0.001). Patients requiring hemofiltration compared to those without had a Δ-SOFA of −1.2±1.4 *vs.* −5.1±3.2 (P<0.001) (*Figure 3B*). Δ-SOFA of patients who deceased in the early postoperative period was at −0.3±1.5 *vs.* −4.8±3.2 (P<0.001) in patients who survived, respectively (*Figure 3C*).

Surgical revisions

Three patients underwent surgical revision one patient after LUTX on ECMO and two patients after lung resection. One CF patient who underwent LUTX newly developed a partial anastomotic dehiscence on POD14 (diagnosed via newly developed fluid pneumothorax on chest X-ray followed by bronchoscopy) that was successfully corrected via right upper lobe lobectomy and reanastomosis of the intermediate bronchus to the right main bronchus. One patient with advanced pulmonary emphysema who underwent lobectomy of the right lower lobe plus decortication of the upper lobe for lung cancer in conjunction with recurrent pleuritis was surgically revised for persistent air leak on POD8. Another patient who underwent lobectomy of the left upper lobe together with anatomic segmentectomy of the segment 6 developed pneumonia in the remaining left lower lobe on POD5 and was surgically revised for narrowing at the site of the left upper lobe bronchial stump via a bronchial sleeve resection.

Mortality

Perioperative (30-day) mortality of the entire study patient cohort was 4.1%. One patient bridged with v/v ECMO

Table 2 Correlation of cytokines with clinical parameters

Parameter	ST2/IL33R	IL-10	IL-6	TNF-α	TGF-β	CRP
Length of ECC						
r	0.262	0.256	0.229	0.023	0.117	0.464
P value	0.045	0.050	0.079	0.859	0.406	0.001
Length of surgery						
r	0.229	0.186	0.122	0.056	0.190	0.155
P value	0.066	0.138	0.331	0.654	0.151	0.269
Max. BL						
r	0.197	0.355	0.217	0.007	0.391	0.318
P value	0.144	0.003	0.076	0.957	0.002	0.017
Min. HB						
r	0.116	0.236	0.115	0.007	0.007	0.025
P value	0.352	0.050	0.454	0.955	0.960	0.854
PRBCs						
r	0.049	0.508	0.351	0.037	0.155	0.177
P value	0.695	0.001	0.003	0.765	0.233	0.854
Fibrinogen						
r	0.068	0.017	0.088	0.015	0.118	0.053
P value	0.585	0.886	0.477	0.906	0.365	0.701
FFP						
r	0.203	0.551	0.419	0.021	0.119	0.225
P value	0.097	0.001	0.001	0.866	0.357	0.095
Noradrenalin						
r	0.104	0.251	0.260	0.181	0.212	0.179
P value	0.402	0.040	0.033	0.137	0.101	0.187

BL, blood lactate concentration; CRP, C-reactive protein; ECC, extracorporeal circulation; ECMO, extracorporeal membrane oxygenation; FFP, fresh frozen plasma; IL, interleukin; Min. HB, minimum serum haemoglobin concentration; PRBCs, packed red blood cells; r, Pearson correlation coefficient; TGF, transforming growth factor; TNF, tumour necrosis factor

to re-transplantation for restrictive allograft syndrome (the initial LUTX was performed for end-stage IPF) that required postoperatively prolonged ECMO support for hemodynamic stability died on POD3 due to concomitant diffuse bleeding and massive central pulmonary embolism. Another patient who underwent LUTX for COPD with an early uneventful postoperative course (extubation POD1, transfer to the normal ward POD3) died on POD 10 due to acute bowel ischemia. One CTEPH patient with a high-risk hemodynamic profile died on POD5 because of persistent pulmonary hypertension after technically successful PEA with resultant prolonged v/a ECMO support.

Discussion

Indications for ECMO support have expanded rapidly beyond acute severe respiratory and cardiac failure and extracorporeal cardiopulmonary resuscitation; to semi-elective procedures, such as bridge to transplantation and elective cardiopulmonary support replacing CPB during

Table 3 Applicability of cytokines to predict postoperative inflammation and organ dysfunction

SOFA	OR	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ST2/IL-33R	2.8	95	80	41	11
IL-10	0.7	89	60	73	8
IL-6	18.6	97	80	82	30
TNF-α	5.5	95	60	78	21
TGF-β	0.1	81	25	25	2
CRP	2.4	95	80	38	11

CRP, C-reactive protein; IL, interleukin; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value; SIRS, systemic inflammatory response syndrome; SOFA, sequential organ failure assessment score; TGF, transforming growth factor; TNF, tumour necrosis factor. Cut-offs were found using the Youden Index of absolute cytokine concentrations at end of surgery for SOFA: IL-10 311 ng/mL, IL-6 69.0 ng/mL, TNF-α 2.3 ng/mL, ST2/IL33R 2,159 ng/mL, TGF-β 17.2 ng/mL and SIRS: ST2/IL33R 1,249 ng/mL, IL-10 82 ng/mL, IL-6 19.0 ng/mL, TNF-α 6.3 ng/mL, TGF-β 18,516 ng/mL, CRP 0.84 mg/dL, SOFA 8.5.

surgery (15,18,20). The main distinction between the acute, semi-elective and elective use of ECMO is the duration of support provided. Acute and semi-elective implanted ECMO can support patients with severe organ failure for weeks and months, whereas elective ECMO is similar to CPB only employed for hours. Semi- elective ECMO as a bridge to transplantation avoids mechanical ventilation and therefore reduces the risk of infection, functional impairment of other organs and muscle deconditioning (18,21). Another important distinction between acute-, semi- and elective ECMO concerns mortality: while mortality rates under acute ECMO range from 37% to 76% depending on the indication, only 10% of all patients who were bridged to transplantation died in the last decade (18,22). Until now, no intraoperative death related to elective ECMO support was reported (16).

In this study we revealed evidence for enhanced Th1 as well as Th2 responses at end of surgery in patients undergoing elective LUTX on ECMO and PEA on CPB, which we did not observe in patients undergoing major pulmonary resections without ECC. The following observations point to an on/off phenomenon concerning SOFA and cytokine expression following major thoracic surgery on ECC support (CPB and ECMO): we did not observe perioperative differences in the quantitative and qualitative cytokine response or SOFA between PEA on CPB and LUTX on ECMO (stressing that no *t*-statistic was employed because of the inherent differences between patient groups and their respective surgery). Concerning the use of ECC the reasons for this on/off phenomenon may lie purely in the contact of blood components with tubing of ECC circuits. Other differences such as the use of an open (venous reservoir during CPB) *vs.* closed circuit (ECMO), no suction of blood in the ECMO system, more bleeding because of full heparinization on CPB, aortic cross clamping during CPB, and others may be of subordinate significance for perioperative inflammation.

Few intraoperative methods were reported to reduce the incidence of inflammation. Beer *et al.* showed that continuous mechanical ventilation during CABG surgery reduced the systemic pro- and anti-inflammatory response (23). In our study, mechanical ventilation was continued during LUTX on ECMO and temporarily discontinued during deep hypothermia during PEA on CPB. Furthermore, pulsatile flow during ECC attenuated the inflammatory response (24). CPB during PEA was performed with non-pulsatile perfusion; whereas various degrees of pulsatile flow were generated by the residual cardiac function of the patient during v/a ECMO, depending on the required respiratory and hemodynamic extracorporeal support (per protocol ECMO blood flow of 50% of the calculated cardiac output). Studies have shown that non-pulsatile perfusion causes a decrease in hemodynamic energy resulting in capillary collapse, microvascular shunting, and activation of inflammatory mediators (25,26).

Our data did not allow differentiating between the effect of ECC and that of the surgical procedure itself as the use of ECMO during LUTX and CPB during PEA remains part of our standard surgical procedures. Since the institutional experience revealed improved short- and long-term outcomes with intraoperative ECMO-support a control group without ECMO could not be provided (18).

Propofol compared to sevoflurane anaesthesia can

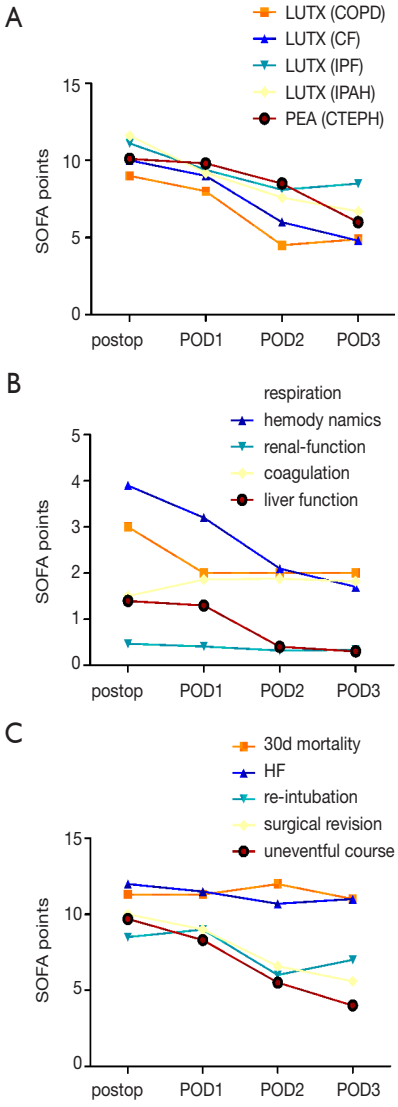

Figure 3 Relative changes in SOFA (Δ-SOFA) in the early postoperative period. A decrease from end of surgery to POD3 of Δ-SOFA in patients admitted to ICU was depicted according to the underlying end stage pulmonary disease COPD, CF, IPF, IPAH and CTEPH patients (A). The postoperative course of the single factors of Δ-SOFA are shown in (B). The postoperative Δ-SOFA of 30-d mortality, patients requiring HF, patients who were reintubated, patients requiring surgical revision and patients with an uneventful postoperative course are shown in (C).

significantly reduce perioperative inflammation and protect pulmonary function (27,28). Sevoflurane was employed in lung cancer (lung resection) and CTEPH (PEA) patients, due to its cardio-protective properties in patients with little or no ischemic heart disease (29). Patients with end-stage pulmonary disease undergoing LUTX were treated with intravenous perfusion of propofol for anaesthesia maintenance to avoid surgeon's exposure to volatile anaesthetic.

Large-scale studies validating the SOFA score in critically ill patients revealed increased mortality (>50%) at a SOFA ≥10 and >95% at SOFA >12 (30,31). In our study only extremely high absolute SOFA ≥13 at end of surgery showed poorer 30-d mortality in the Kaplan Mayer survival analysis. Since perioperative mortality in our ICU patient cohort with LUTX on ECMO and PEA on CPB patients was very low (3 out of 57 patients, 5%), absolute SOFA scores may pertain to treatment strategies rather than real organ failure: the SOFA score assesses hepatic function only by determining total bilirubin levels. In a study on open-heart surgery transient hyperbilirubinemia was evident by one third of all patients due to increased hemolysis caused by CPB (32). In our cohort continuous hemofiltration is frequently performed for excessive fluid overload removal in the presence of near normal renal function. In these patients, treatment strategies might change the renal SOFA score (creatinine levels and urine output) without genuine alterations in renal function. Further, CPB is known to lower platelet count in the early postoperative period, thus affecting the SOFA score by itself (33). In this study, we observed the same phenomena in patients undergoing LUTX on ECMO and PEA on CPB. Besides, SOFA has some limitation in actively sedated patients due to the use of the Glasgow coma scale to assess neurological function. Therefore, we customized the score by excluding the neurological assessment completely.

In patients undergoing cardiac surgery Δ-SOFA varied among −3 to +1 according to length of ICU stay (33). In contrast, in our study Δ-SOFA calculated from end of surgery to POD3 ranged from −3.9±3.3 to −5.5±2.5. The Δ-SOFA was significantly smaller in patients requiring re-intubation, hemofiltration or experiencing early mortality. Since SOFA is only applicable to ICU patients, the clinical course of patients transferred to the normal ward before POD3 was not monitored by Δ-SOFA. Therefore, Δ-SOFA did not capture the patients with the best postoperative course and might underestimate the difference in Δ-SOFA between patients with a complicated and an uncomplicated course. Patients undergoing lung resection were not admitted to ICU. Therefore, the qSOFA was used to detect organ failure (7). None of the patients after lung resection without ECC met the qSOFA criteria.

In our investigation, IL-6 was the most sensitive

parameter for detection of SOFA. IL-6 was reported to be a highly sensitive mediator of the acute phase reaction, allowing inflammatory conditions to be detected before the onset of associated clinical symptoms or before a rise in CRP (34). SOFA and cytokine release were phenomena irrespective of PGD after LUTX.

The chronological timeline of our measured cytokines IL-10, IL-6, ST2/IL33R and TNF-α were in line with previous findings of patients undergoing cardiac surgery (35,36). Another previous study showed that IL-10 rose significantly in patients undergoing on-pump compared to off-pump CABG surgery (37). In our study, ECC-time correlated significantly with IL-10 and ST2/IL-33R concentrations, irrespective of total length of surgery. Further, serum concentrations of IL-10 prior to surgery were significantly increased in patients with semi-elective v/v ECMO bridging compared to non-bridged patients.

Immunosuppression is an important part of ensuring allograft lung function after LUTX (38). Patients undergoing LUTX with elective ECMO received 1,000 mg methylprednisolone 30 minutes prior to allograft reperfusion. After surgery three stages of immunosuppression followed: induction, maintenance, and treatment of acute rejection. Patients undergoing PEA received 100 mg hydrocortisone prior to initiation of CPB in order to attenuate SIRS induced by CPB. Several trials investigated the use of steroids as a cheap method to mitigate the inflammatory response caused by CPB. The DECS trial included 4,494 patients requiring cardiac surgery with CPB. Randomization of patients to intraoperative dexamethasone 1 mg/kg or placebo did not result in different outcomes. However, subgroup analysis focusing on patients with a EuroSCORE >5 demonstrated significant reductions in infection, delirium, and death under dexamethasone (39).

While immunosuppressive effects of PRBC transfusion were implied by reduced organ rejection after renal transplantation, subsequent studies observed pro-inflammatory effects and worse outcomes of LUTX in response to large-volume PRBC and platelet transfusion (40-42). In our study the number of transfused PRBC correlated positively with anti-inflammatory serum IL-10. Prophylactic FFP transfusion to critically ill non-bleeding patients resulted in decreased TNF-α levels (43). In our study, the amount of FFP transfusion correlated positively with IL-10 serum concentrations, maximal lactate levels and ECC-time.

Innate immune cells produce high amounts of lactate during inflammatory activation (44). *In vitro* treatment with lactate >24 h of peripheral blood mononuclear cells from healthy donors significantly modulated cytokine production with predominantly anti-inflammatory effects (45). We demonstrated a strong correlation between anti-inflammatory IL-10 and maximum lactate levels at end of surgery.

Our prospective, study has several limitations pertaining to its observational design. Hypothesis testing between the three patient groups: LUTX on ECMO, PEA on CPB and lung resection for cancer was not performed because of the inherent differences between the groups. Consequently, no statistical comparisons between the usage of ECMO and CPB can be drawn. The institutional LUTX experience clearly showed better outcomes with intraoperative ECMO support, since controlled reperfusion of a newly implanted pulmonary graft over a time period of 5 to 10 minutes saves the vulnerable organ. However, controlled reperfusion can only be achieved by using CPB or ECMO support. While, the potential beneficial effect of CPB during LUTX is hindered by an augmented intraoperative blood turnover and an increased risk of postoperative bleeding, ECMO support seems the best option to provide controlled reperfusion without increasing those risks (46,47). Thus, no concurrent control groups of patients undergoing LUTX without intraoperative ECMO support could be provided (16,18). Therefore, limitations concerning different surgical and anaesthesiological strategies for patients undergoing LUTX (ECMO), PEA (CPB) and lung resection (no ECC) are obvious and detailed in the manuscript. Moreover, patients undergoing LUTX received postoperative immunosuppression. However, specific immunosuppression pertaining to LUTX was launched after the second blood draw (at end of surgery). Therefore, only cytokine levels from POD1-POD5 were altered due to immunosuppression strategies in patients undergoing LUTX. Further, there is obvious unavoidable bias regarding the age and comorbidities of our cohort. Patients with COPD, IPF, CTEPH and lung cancer were by far older than CF and IPAH patients receiving bilateral LUTX. Patients with IPF are more likely to require ECMO bridging to transplant.

Conclusions

Elective thoracic surgery on ECC support followed by an uneventful postoperative course and excellent outcomes triggered an immediate rise and concomitant fall of inflammation as observed in serum cytokine release and SOFA criteria. High absolute SOFA scores in the presence

of an uncomplicated postoperative course may pertain to specific management strategies rather than organ failure. IL-6 serum concentrations, extremely high SOFA ≥13 and a missing decline in Δ-SOFA may predict outcomes. Future studies investigating the potential biological significance of perioperative cytokine release are warranted.

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CHAPTER THREE: DISCUSSION

3.1 General Discussion

In this research work we found an increased Th1 and Th2 response following extensive thoracic surgery in LUTX patients on elective ECMO and PEA patients on CPB, but not in patients undergoing lobectomy without ECC. Clinically and experimentally detected inflammation shown as high SOFA and enhanced cytokine expression after extensive thoracic surgery on ECC support direct toward an on/off phenomenon. Thus, the contact of blood to the synthetic non-degradable membranes of the ECMO or CPB circuits may be responsible for this inflammatory on/off phenomenon. The quantitative and qualitative cytokine "storm" did not differ between PEA and LUTX patients. The inflammatory response seems therefore unaffected by discrepancies between CPB and ECMO support such as the open venous reservoir during CPB or the closed circuit during ECMO, suction of blood in the CPB, aortic cross clamping during CPB, enhanced hemodilution during CPB, hypothermia or more bleeding due to full heparinization on CPB.

Heparin is a well-known anticoagulant, showing protective anti-inflammatory properties by binding to histones and thereby reducing histone induced pro-inflammatory cytokines including IL-6 and IL8, tissue factor and C3a.[382, 383]It has been reported that the anti-inflammatory and anticoagulant properties of heparin, which is a highly negatively charged polysaccharide, are induced by bonded sulphate groups.[384] In a study on sepsis patients heparin treatment has been considered as a treatment strategy beyond anticoagulation.[385] However, bleeding was noticed as a considerable drawback for heparin treatment as a sole anti-inflammatory agent, since higher dosages are required to be effective for this indication. Hogwood and colleagues investigated whether desulfated heparin can reduce anticoagulant activities. They found that selectively desulfated heparins have reduced anticoagulant effects, while the activity as an anti-histone agent remained. Unfortunately, fully desulfated heparins were ineffective as an anti-inflammatory agent.[383] In our study heparin was administered solely for anticoagulation. Prior to induction of both ECMO and CPB unfractionated heparin had to be administered. At the ICU low molecular weight heparin was continued for prophylactic anticoagulation. Prior to ECMO start a priming dose of 60 IU/kg of unfractionated heparin was administered. In contrast, prior to CPB initiation full-heparinization with 400 IU/kg of unfractionated heparin was required. Nevertheless, we did not identify reduced pro-inflammatory or increased anti-inflammatory cytokines due to different anticoagulation regimen among both groups.

Beer and colleagues published in several research works that continuing mechanical ventilation can reduce the systemic pro- and anti-inflammatory response during CPB in patients undergoing heart surgery.[106] In this study, mechanical ventilation was continued during ECMO in LUTX patients but discontinued during CPB in PEA patients. Besides, pulsatility during ECC is known to reduce inflammation.[386] In contrast, non-pulsatile blood flow activates inflammatory mediators in response to reduced hemodynamic energy resulting in capillary collapse and microvascular shunting. [173, 387] In our study CPB employment during PEA was performed without pulsatile perfusion. Contrary, during LUTX on ECMO pulsatility was generated by the persistent cardiac function according to the required respiratory and hemodynamic ECC support. In a study on 62 lung cancer patients undergoing lobectomy total intravenous anaesthesia (TIVA) with propofol significantly reduced perioperative inflammation compared to volatile anaesthesia with sevoflurane. Patients receiving TIVA had significantly lower IL-6 expressions and higher IL-10 serum levels. [388, 389] In this research work we employed sevoflurane for patients undergoing lobectomy and PEA according to our institutional standards. Studies on patients with and without ischemic heart disease found that sevoflurane has cardio-protective properties.[390] In patients undergoing LUTX we performed a TIVA according to our institutional standards to avoid surgeon's exposure to volatile anesthetics.

The use of ECMO expanded widely over the years, from acute indications during respiratory and cardiac failure to semi-elective bridging to transplantation and elective cardiopulmonary support instead of CPB during major thoracic procedures.[391-393] The main distinctions between acute, semi-elective and elective ECMO support concerns the duration of time provided. While patients with acute organ failure on acute or semi-elective ECMO may require support for weeks or even months, elective ECMO during surgery only remains implanted for hours.[166] In the COVID-19 pandemic acute and semi-elective ECMO support became a feasible option in COVID 19 patients who did not respond to conventional therapeutic interventions to either bridge patients to LUTX or to recovery.[394, 395, 396] Bridging strategies have the advantage to avoid intubation and mechanical ventilation, thereby reducing the risk of those critically ill patients to acquire infections and especially muscle deconditioning.[156, 391] Furthermore, there are differences concerning mortality between acute-, semi- and elective ECMO indications. Under acute, in contrast to semi-elective ECMO the mortality rate ranges from 37% to 76% compared to 10%.[391, 397] Regarding elective ECMO, no intraoperative death was published until now.[162]

Studies reported that a SOFA ≥10 and SOFA >12 is related to a mortality of more than 50% and 95%. [398, 399] In this study, only very high SOFA≥13 at ICU admission were predictive for increased 30 day mortality. We concluded that absolute SOFA might rather refer to therapeutic strategies than demonstrate real organ dysfunction. SOFA evaluates each organ system separately by assigning 0-4 points for hepatic, respiratory, coagulation, cardiovascular, neurological and renal function.[400] SOFA employs total bilirubin levels to assess hepatic function. In a study on cardiac patients transient hyperbilirubinemia was reported in up to one third off patients. The authors concluded that hyperbilirubinemia may was induced by haemolysis during CPB.[401] Similarly, increased bilirubin levels due to haemolysis and liver hypoperfusion were reported in ECMO patients. In a study on 89 patients with cardiac disease receiving va ECMO hyperbilirubinemia (>3 mg/dL) was even reported in 73% of all included patients.[402, 403] Bilirubin is the end product of heme catabolism. The liver metabolizes plasma free haemoglobin levels to bilirubin; plasma free hemoglobin levels increase due to pump head or oxygenator thrombosis, insufficient anticoagulation and destroyed blood reed cells by excessive pump speed (>3000 rpm).[404] Another reason for higher bilirubin levels in ECMO compared to CPB patients may lie in prolonged periods of ECMO usage. [402] In our ICU cohort 9 % of all patients had bilirubin levels <1mg/dl, 55% between 1-3mg/dl and 36% had bilirubin serum concentrations >3mg/dl. Further, a study on 836 ICU patients investigated that SOFA has a high accuracy to predict for 28d and 90d mortality in patients with acute kidney injury requiring continuous renal replacement therapy.[405] Contrary, in our cohort continuous hemodialysis was frequently applied in the absence of renal failure, but to treat excessive fluid overload. In these patients, renal SOFA might have been biased by management strategies. Additionally, CPB is known to subsequently lower platelet count. Accordingly, ECMO support impaired platelet aggregation and platelet activation already after one day of in 33 adult patients in Denmark, thus ECMO and CPB usage affects the SOFA already by itself.[406] [407] While SIRS is counting to the main causes for thrombocytopenia in ICU patients occurring in up to 48% of all patients, it remains important to evaluate other pharmacological and clinical etiologies for reduced platelet count in ICU patients. Heparin is the most important differential diagnosis with regard to drug-induced thrombocytopenia. Heparin-induced thrombocytopenia (HIT) occurs due to the development of IgG antibodies against complexes of platelet factor-4 and heparin.[408] The diagnosis of HIT should be considered when the platelet count falls below 50 G/L (or by 50% from baseline) after day 5 and 14 after exposure to any

heparinoid product.[409, 410] In our patient cohort we did not diagnose HIT. In the literature HIT was reported to occur in more than 8.3% of all patients undergoing cardiac surgery on CPB and in less than 1% of all patients who require ECMO support.[411, 412] We did not identify differences regarding SOFA in LUTX compared to PEA patients. Due to several limitations regarding the SOFA of neurological function in actively sedated surgical patients, we adjusted the scoring system by excluding neurological assessment entirely. Additionally we analyzed the postoperative time course of SOFA by calculating Δ-SOFA from end of surgery to POD3 ranging from -3.9±3.3 to -5.5±2.5. In cardiac patients Δ-SOFA was reported to range from -3 to +1 according to the duration of time at the ICU. [406] In this study we correlated decreased Δ-SOFA with early complications such as re-intubation, hemofiltration and 30 day mortality. Δ-SOFA did not monitor patients with an uncomplicated postoperative course who were transferred to the normal ward during the first 3 days. Therefore, we assume that the difference in Δ-SOFA between patients with complicated and uncomplicated postoperative time course was underestimated in the present cohort study. Additionally, all patients after lobectomy were transferred to the normal ward. In the normal wards patients were monitored with on a routine basis.[413] However, none of our patients who underwent lobectomy had a positive qSOFA.

During acute phase reactions IL-6 is known as a highly sensitive cytokine, enabling to detect inflammatory states before the beginning of related clinical symptoms. [414] In this study, IL-6 was the most sensitive mediator to detect clinical inflammation, measured as high SOFA. Regarding outcome after LUTX, PGD is significantly associated with increased 30- and 90-day mortality, with a stepwise increase with PGD severity.[185, 186] In contrast, we did not find correlations between perioperative inflammation demonstrated via SOFA and cytokine concentrations and PGD scorings.

In line with previous results of patients undergoing cardiac surgery we found a similar time-course of the investigated cytokine levels comprising IL-6, ST2/IL33R, TNF-alpha and IL-10. [87, 103] IL-10 and ST2/IL-33R levels correlated significantly with duration of ECC, independent of total length of the procedure. Additionally, at baseline IL-10 was significantly higher in patients who were bridged to transplantation via ECMO. These findings emphasized previous results of patients undergoing on-pump CABG surgery. In these patients IL-10 was significantly increased compared to patients undergoing off-pump CABG surgery. [101]

Immunosuppression is essential to guarantee allograft lung function after organ transplantation.[437] In our cohort study 1000mg of methylprednisolone was administered to LUTX patients 30 minutes before allograft reperfusion. After surgery LUTX patients

received a triad of immunosuppressive therapy including induction, maintenance and treatment of acute rejection. In contrast, patients undergoing PEA received hydrocortisone 100 mg prior to the start of CPB to attenuate CPB support-induced SIRS. In patients undergoing organ transplantation Immunosuppression plays a decisive role to ensure allograft lung function.[415]The application of steroids as a cheap option to attenuate the inflammatory response of the ECC circuits during cardiac surgery has been investigated intensively. One of the largest trials randomized 4494 cardiac patients on CPB into an intraoperative dexamethasone and placebo group. However, there was no difference concerning outcome between both groups. Solely in a subgroup analysis of high-risk patients with an European system for cardiac operative risk evaluation (EuroSCORE) >5 they found significantly mitigated infection rates, onset of delirium and death under dexamethasone therapy. [416] Patients with an EuroSCORE>5 had a predicted in-hospital mortality after cardiac procedures of more than 10% and are categorized as high risk patients.[417] During cardiac surgery on CPB Bernardi and colleagues investigated the usage of perioperative hemoadsorption via the CytoSorbTM adsorber to eliminate serum cytokine levels in those patients. They did not find any differences of pro-inflammatory cytokine levels between both groups, but absolute serum concentrations were reduced within the first 24 hours after CPB.[418] Massive transfusion of PRBC is necessary in one of four patients undergoing LUTX due to following causes. [419, 420] 1) Patients requiring LUTX as a consequence of end-stage COPD often present preoperative polycythaemia induced by chronic hypoxia; In these patients oxygen delivery is dependent on high normal hemoglobin levels. Therefore it is important to increase the transfusion trigger in those patients to maintain higher hemoglobin levels during the entire perioperative period. 2) Contrary, CF patients are often already anaemic prior to surgery due to chronic infections. In those patients already little bleeding results in PRBC transfusion. 3) Massive bleeding and thermodynamically unstable situations appear frequently during LUTX, inducing transfusion. 4) Modern transplantation techniques are performed without bronchial artery revascularisation, resulting in solely donor lung perfusion via the pulmonary circulation with lower oxygen content. As a result, increased haemoglobin levels become necessary to guarantee sufficient tissue oxygenation to avoid graft dysfunction and organ failure. [421, 422] Therefore, our institution performs a more liberal transfusion policy of PRBC using an increased transfusion threshold of haemoglobin <10g/dl to avoid severe intraoperative anemia. Menger and colleagues reported in LUTX patients an association between increased postoperative hemoglobin levels and improved one-year survival rate. Assuming that higher hemoglobin levels after LUTX improve graft function and avoid

systemic hypoxemia. [423] PRBC transfusion was reported to reduce organ rejection after renal transplantation due to immunosuppressive effects. In contrast, further studies observed pro-inflammatory effects and adverse outcomes after LUTX following massive transfusion of PRBC and platelets. [424-426] IL-10 serum concentrations and PRBC and FFP count correlated positively. Furthermore, FFP transfusion correlated with ECC-time and maximum lactate levels. Fibrinogen is an acute phase protein, which is synthesized by the liver and exerts coagulatory and pro-inflammatory properties. In severe inflammatory conditions the plasma concentration of fibrinogen can increase up to four-fold, from baseline. [427] In contrast, physiologic concentrations of fibrinogen remain sufficient for coagulation. [428, 429] In our study, fibrinogen serum concentrations decreased below baseline post surgery and increased in the following days thereafter beyond baseline levels. Therefore, fibrinogen followed a contradictory pattern compared to the investigated cytokine serum concentrations. Low plasma fibrinogen concentrations are known to occur frequently after cardiac surgery on CPB, due to hemodilution.[430-432] In the early postoperative period normal or even supranormal fibrinogen levels are regularly observed inducing a prothrombotic state.[433, 434] Our findings emphasize that CPB and ECMO induce hemodilution, known as a major contributor of perioperatively occurring hypofibrinogenemia. Serum lactate concentrations are used as a surrogate for tissue hypoperfusion and organ dysfunction during times of anaerobic metabolism.[435] It has been reported that innate immune cells produced high lactate levels during inflammatory conditions. [436] IL-10 serum concentrations and maximum lactate levels correlated positively.

3.2 Limitations of this study

Our prospective cohort study is limited by its observational design. Hypothesis testing between LUTX, PEA and lobectomy patients was not performed due to large, aetiological, surgical and anaesthetic differences between the 3 groups. Our institutional experience found improved short- and long-term outcomes in LUTX patients on intraoperative ECMO. Therefore, it would have been unethical to form a control group of patients undergoing LUTX without ECMO.[160 162, 391] As a result, we were unable to distinguish between the inflammatory reaction of the ECC circuit and the surgical procedure itself. Besides, elective intraoperative ECMO support enables controlled and slow reperfusion of the newly implanted vulnerable pulmonary allograft. The advantage of CPB employment during LUTX seems reduced by an elevated blood turnover during surgery and an augmented risk of postoperative bleeding. In contrast, ECMO support guarantees

controlled reperfusion without increasing bleeding complications. [153, 165] Further limitations concern the postoperative immunosuppressive regimen after LUTX. However, immunosuppressive treatment was started after ICU admission, therefore relevant effects can only occur after the second blood draw. Therefore, the first two measuring points were not influenced by immunosuppression. Additionally there were major differences concerning age and comorbidities between COPD, IPF and CTEPH compared to CF and IPAH patients.

3.3 Conclusion

In Summary, perioperative ECMO and CPB support induce an immediate elevation and concomitant fall in pro- and anti-inflammatory cytokines, as detected in serum cytokine concentrations and clinically by high SOFA; without affecting the postoperative course of these patients who underwent elective extensive chest surgery. The anti-inflammatory compared to the pro-inflammatory immune response was even more pronounced in our patients. Further, our findings point out that high absolute SOFA together with an uneventful postoperative outcome may highlight specific treatment strategies rather than identifying patients with organ dysfunction. However, very high serum IL-6 concentrations and SOFA ≥ 13 and no decrease in Δ-SOFA can predict an unfavourable short-term outcome. Future investigations on the clinical significance of perioperative cytokine concentrations remain warranted.

3.4 Outloook – Nitric Oxide

In 2004 Gianetti and his study group established the hypothesis that administering NO during cardiac surgery reduces the ischemia-reperfusion injury. Therefore, they examined the biochemical effects of 20 ppm of iNO in patients undergoing heart surgery on CPB. The authors found that low dose iNO significantly reduced the release of biomarkers specific for myocardial injury and concluded that the anti-inflammatory properties of NO could partly mediate cardiac protection when administered via inhalation.[437] More recently, 2 paediatric and 2 adult single- center trials evaluated the use of NO delivered at 20 ppm into the CPB circuit.[438, 439] In the first trial on 16 children NO significantly lowered the time of invasive ventilation and the time at the ICU and lowered postoperative troponin levels.[440] The second trial investigated 198 children and showed a reduced rate of low cardiac output syndrome in children younger than 2 years, with the most marked in children younger than 6 weeks.[438] Another trial on 60 adult patients undergoing CABG

reported lower postoperative cardiac enzymes and vasopressor use in patients randomized to NO delivered at 40 ppm into the CPB cirucit.[438] Further, a study on 244 patients who underwent valve replacement surgery found a lower incidence of acute kidney injury in the NO group, delivered at 80 ppm into the CPB followed by 24 hours of iNO.[439] These low evidence trials stand in variance with the largest double-blind multicentre randomized clinical trial published in July 2022. This study enrolled 1364 children younger than 2 years undergoing CPB surgery for congenital heart disease receiving either NO delivered into the CPB or standard care. In this study they found no significant difference in the number of ventilator-free days, the incidence of low cardiac output syndrome or postoperative ECLS within 48 hours after initiation of CPB and death within 28 days.[441]

Nonetheless, the first attempts regarding the application of NO during ECMO have been taken. The incorporation of NO into ECMO circuits has been recently shown to be possible and safe.[442] However, NO application during ECMO has some further risks: NO comes at a concentration of 800 ppm, to apply 20 ppm, a total fresh gas flow of at least 3 litres per minute is therefore required. This is easily achieved on CPB, with the possibility to add CO_2. During ECMO support 3 litres per min of gas flow can lead to a high pressure on the gas side of the membrane and thereby leading to gas embolism.[442] In 2018 a small observational trial by Chiletti and colleagues compared the outcomes of 30 paediatric patients on ECMO who received NO into the ECMO circuit compared to 101 patients who received standard ECMO care. The use of NO during ECMO was found to be safe, with median levels of methaemoglobin within normal ranges, despite the long duration of administration of NO compared to CPB.[443] The occurrence of methaemoglobinemia after NO administration remains one of the major safety concerns. Due to the oxidative potential of NO, it can react with Hb to form metHB.[444]

Our study group remains interested to further investigate whether NO blunt the perioperative immunoreaction in patients undergoing LUTX and if NO administration may help us to improve patient's outcome.

CHAPTER FOUR: MATERIALS & METHODS

The following information has been essentially reproduced verbatim from my publication "Transient perioperative inflammation following lung transplantation and major thoracic surgery with elective extracorporeal support: a prospective observational study" in 2021.

4.1 Ethical approval

The present study was performed in accordance with the Declaration of Helsinki (as revised in 2013). Approval was obtained by the Institutional Ethics Committee of the Medical University of Vienna (EK1363/2018). Written informed consent was obtained from all study participants including patients and volunteers.

4.2 Study design, setting and patients

This prospective cohort study was designed as a purely observational investigation at the Medical University of Vienna. The study was composed as a translational project, combining clinical and experimental research. During a period of 12 months (from May 2018 until April 2019) forty-two patients with end-stage pulmonary disease {COPD (n=15), CF (n=15), IPF (n=7), IPAH (n=5)} undergoing LUTX on ECMO, fifteen consecutive Chronic Thromboembolic Pulmonary Hypertension (CTEPH) patients undergoing PEA on CPB and 15 consecutive lung cancer patients undergoing major lung resections without ECC were consecutively included. Pregnant women, patients who were younger than 18 years and patients who did not give written informed consent were excluded. In total 6 blood samples were drawn from all participating patients: before surgery (baseline), at ICU admission, once on each of the 3 following post- operative days (PODs) and at POD5. Cytokine serum concentrations were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits. We analysed the perioperative course of IL-6, IL-10, ST2/IL-33R, TNF-alpha and Transforming Growth Factor(TGF)-β serum concentrations.

4.3 Sandwich ELISA technique

According to the manufacturers` instructions we measured IL-6, IL-10, ST2/IL-33R, TNF-alpha and transforming Growth Factor (TGF)-β serum concentrations by commercially available enzyme- linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, Minnesota, United 130States). We incubated 96-well microplates with capture antibodies

(Mouse anti-human IL-6, IL-10, ST2, TNF-alpha and TGF-β) overnight at room temperature and used assay buffer for blocking. Following incubation of serum samples and washing, HRP-conjugated detection antibodies were added (Biotinylated goat anti-human IL-6, IL-10, ST2- TNF-alpha and TGF-β). A colour reaction was obtained after inclusion of peroxidase reagent tetramethylbenzidine (TMB) (Sigma-Aldrich Corp., St. Louis, MO, USA). An absorbance microplate reader for ELISA, the Infinite F50 (Tecan, Männedorf, Switzerland) read the optical density (OD) at 450 nm and 540nm.

4.4 Perioperative management

4.4.1 Anaesthesia

All patients enrolled in this study received standard perioperative monitoring according to our institutional standards after entering the operating room. Anesthesia induction was performed using 2 mg Midazolam, Fentanyl 0.2 µg/kg, Propofol 2 mg/kg and Cis-Atracurium 0.2 mg/kg for CETPH and patients with end-stage pulmonary disease undergoing PEA and LUTX and Rocuronium 0.5 mg/kg for patients with lung cancer undergoing lung resection. Antibiotic prophylaxis was administered in accordance with our institutional guidelines: PEA and LUTX patients received Piperacillin/tazobactam 4.5 g and patients undergoing lung resection obtained cefuroxime within 30 to 60 minutes before incision. In patients undergoing PEA and LUTX a Swan-Ganz catheter and a central venous catheter were placed into the right or left jugular vein to measure pulmonary artery pressures. For anaesthesia maintenance total intravenous anaesthesia was performed in LUTX and PEA patients using fentanyl 200ug/h and propofol 6 mg/kg/h, whereas volatile anaesthesia was administered in patients undergoing lung resection using sevoflurane with a minimal alveolar concentration of 1. Transesophageal echocardiography was installed in patients undergoing PEA and LUTX to monitor cardiac pump function, volume status and adrenergic support during surgery. In LUTX patients an initial dose of 60 IU/kg of heparin was administered prior to intraoperative va ECMO initiation. In contrast, PEA patients undergoing PEA received 400 IU/kg of heparin before CPB start. Normothermic conditions of 37°C were maintained in patients undergoing LUTX and lung resection. Patients undergoing PEA were cooled to 18°C to guarantee deep hypothermia during the periods of complete circulatory arrest.

4.4.2 Surgery

Donor lungs were harvested during multi-organ procurement and offered to recipients according to their ranking on the match list following the guidelines of Eurotransplant. To estimate each candidate's medical urgency prior to transplantation and the probability of success after transplantation the LAS was used. The patient with the highest LAS became first to receive a lung offer. Harvested lung allografts were preserved with colloid containing low potassium solution and kept inflated during transport. LUTX was performed through either bilateral thoracotomy or clamshell incision. Intraoperative central va ECMO was applied in all patients. ECMO prolongation into the postoperative period was performed in hemodynamic and/or respiratory unstable patients. In patients requiring ECMO prolongation, central cannulation was switched to the peripheral femoro-femoral location.[162] In all CTEPH patients undergoing PEA surgery was initiated through a median sternotomy. CPB was cannulated according to our institutional standards. After aortic cross-clamping, cardioplegia was administered. Further, patients were cooled down to 18°C to achieve deep hypothermia to ensure 2 periods of circulatory arrest for improved visualization of the pulmonary arteries and enable the surgeon to perform sequential bilateral PEA.[234] In patients with cancer undergoing lung resection muscle-sparing antero-lateral thoracotomies were performed. In this study we included only patients undergoing anatomic pulmonary resection (lobectomy/pneumonectomy) during single lung ventilation (double-lumen intubation).

4.5 Extracorporeal circulation

Bridging strategies were performed according to patient's hemodynamic and respiratory stability. Patients with severe hypoxic or hypercapnic respiratory failure received v/v ECMO support (Cardiohelp, Oygenator Quadrox)/(Xenios, Oxygenator Hilite) with a 2-site (femoro-jugular) or single-site large-bore double-lumen cannula ranging from 27F to 31F (Avalon Laboratories, Los Angeles, Calif). [391] All patients undergoing LUTX were transplanted on intraoperative, central heparin-bound va ECMO (Medtronic Carmeda), hollow-fiber oxygenator (Medtronic), centrifugal pump (Biomedicus), flow probes and 3/8-inch internal diameter according to our institutional standard. [160]In PEA patients priming of the CPB circuit was performed with 1000 ml crystalloid, 500 ml colloid solution, 5000 IE heparin and 100 ml mannitol 20 %. CPB was achieved using non-pulsatile flow at $2.5\ l \cdot min^{-1} \cdot m^{-2}$, a non-heparin-coated circuit, and a membrane oxygenator (Quadrox™, Maquet, Hirrlingen, Germany, or Capiox, Terumo, Eschborn, Germany). [418]In patients

requiring ECMO prolongation a femoro-femoral v/a ECMO was inserted using a 17F to 19F drainage and 15F to 17F reperfusion cannulas (all Bio-Medicus Cannula, Medtronic Inc, St Paul, Minneapolis). Anticoagulation during prolonged ECMO support was obtained using subcutaneous administration of low-molecular-weight heparin, monitored with daily antiXa measurements. ECMO prolongation was continued until hemodynamic and respiratory stability was achieved, patients had normal chest X-rays, low ventilation patterns and a normalized fluid balance.

4.6 Postoperative management

Induction therapy was administered in 87% of all patients undergoing LUTX directly after ICU admission. Eighty-four percent received alemtuzumab, 3% Anti-thymocyte globulin and in 13% no induction therapy was applied. Maintenance therapy was employed in all patients comprising tacrolimus, mycophenolat-mofetil (only in patients without induction therapy) and corticosteroids. Postoperative antiinfectious prophylaxis therapy was administered in all patients including piperazillin/tazobactam, lifelong Pneumocystis prophylaxis with trimethoprim-sulfamethoxazole, prophylactic inhalation therapy with amphotericin B and gentamicin; and CMV prophylaxis including CMV hyperimmuno-globulines (POD 1,7,14 and 21) together with valganciclovir. Patients undergoing PEA received either piperazillin/tazobactam or cefazolin for postoperative antibiotic prophylaxis. Lung cancer patients undergoing lung resection did not routinely receive postoperative antibiotic therapy.

4.7 Primary Graft dysfunction

According to the ISHLT consensus definition of PGD 2017 patients were classified according to the degree of multifactorial lung injury in the first 72 hours after LUTX. [184] Patients on prolonged postoperative ECMO were classified as PGD Grade 3 when pulmonary infiltrates on chest x-ray were present, whereas those without pulmonary infiltrates as PGD ungradable. [445]

4.8 Definition of SOFA

To evaluate organ dysfunction SOFA was applied once daily in all patients admitted to the ICU including patients after LUTX and PEA. 0-4 points were assigned for each organ system according to respiratory, coagulation, hepatic, cardiovascular and renal function**.** The neurological assessment by employing the Glasgow coma scale (GCS) was excluded

due to limited evaluability in actively sedated patients. Clinical data was collected to perform SOFA with exclusion of neurological function comprising the need for mechanical ventilation, arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) ratio, platelet count, bilirubin, mean arterial pressure, doses of adrenergic agents, creatinine and urine output.[400]

4.9 Statistical Analysis

In this study normally distributed data was presented as mean ± SD, non-normal distributed data was reported as median (range). Mann-Whitney U test was employed for independent, the Wilcoxon matched-pairs signed rank test for dependent non-normally distributed data, the Kruskal Wallis test was used for multiple testing of non-normally distributed data and *t* tests were used for parametric data. To analyze the strength of the linear relationship between cytokine concentrations and clinical parameter Pearson's correlation coefficient *r* was employed. Further, we calculated the Youden Index to identify optimal cut-offs of cytokine serum concentrations to perform outcome analysis. We therefore plotted the receiver operating characteristic(ROC) curve. Binary logistic regression was calculated to evaluate the negative predictive value (NPV), positive predictive value (PPV), sensitivity, specificity and the OR to predict postoperative SOFA (>10 points). Further, Chi-square tests were used to test relationships between categorical variables. Survival analysis was performed using Kaplan Mayer analysis. The level of statistical significance was set at 0.05 (two-tailed p-values). We used SPSS software(version 25; IBM SPSS Inc., IL, USA) and GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA) for statistical analyses and visualization. Boxplots were used for graphical depiction and were designed as followed: Box: 1st to 3rd quartile, Bar: median, Whiskers: percentile 5-95, Outliers: all shown as dots.

PART II - Alpha-Gal specific Immune response after transcatheter aortic valve replacement

CHAPTER FIVE: INTRODUCTION

5.1 Alpha-Gal

In 1965 the glycolipid, Galα1.3–Galβ1–4GlcNAc–R (alpha-Gal) was described for the very first time.[446] Soon thereafter, alpha-Gal was discovered on various cell types of different animals including cows, pigs, sheep, rabbits and rats, however not in human beings and old world monkeys.[447, 448] The alpha-Gal epitope is now determined as an integral carbohydrate of the gycokalyx of various cell surfaces, in particular endothelial cells of bacteria and all mammals expecting primates.[13, 449] In the trans-Golgi network synthesis the alpha-Gal epitope is catalysed by the membrane bound enzyme, known as β–galactosyl alpha 1– 3–galactosyltransferase.[450] In 1984, Galili et al. found high specific IgG antibody titers against alpha-Gal in humans and therefore concluded that there must be an intense, constant antigenetic stimulation throughout life. [450] Next to humans, anti-Gal is present in Old World monkeys and apes in comparable titers, however absent in New World monkeys.[451] In the following years, beside anti- alpha-Gal IgG, IgM and igA antibodies were discovered, accounting for up to 1-2% of all circulating antibodies.[452, 453] Since there is no existing immune tolerance to the alpha-Gal epitope in humans, Galili and colleagues explained the high concentration of anti alpha-Gal ABs by constant antigenic stimulation of bacterial strains of the commensal human intestinal flora, which present alpha Gal epitopes. The study group found interactions between anti-Gal ABs and isolated bacteria of the stool including Escherichia coli, Klebsiella and Salmonella stains by the use of direct immunostaining assays and ELISAs.[13] Beside alpha-Gal bearing bacteria, some viruses also influence the anti-Gal AB production such as the human papillomavirus and the human influenza virus, which are both carrying the alpha-Gal epitope.[454, 455] Therefore, viruses are further discussed as an important stimulant for the production of the huge amount of xenoreactive ABs in humans.[456] The alpha-Gal epitope has similarities to the blood group antigen A and B. Conversely anti-Gal antibodies are closely related to AB0 antibodies. Again Galili and his co-workers discovered that the spectrum of anti-Gal specificity is dependent on the individual's blood type. Thus, B and AB type individuals produce anti-Gal ABs that only bind to the alpha-Gal epitope. In contrast, A or O type individuals who lack the B epitope on their red cells have clones of

anti-Gal ABs that bind to both, alpha-Gal and the B antigen. These findings were shown by immunochemical and hemoagglutination analysis. In addition, adsorption experiments to remove anti-Gal ABs indicated that the majority of the antibodies designated anti-B are in fact anti-Gal clones that can also interact with the alpha-Gal epitope.[457] Besides, Galili and his study group demonstrated that anti-Gal IgG ABs bind to senescent erythrocytes, thereby enabling phagocytosis of these aged cells.[458]

5.2 Alpha–Gal and Xenotransplantation

Transplantation is well described as one of the last therapeutic option for patients with end-stage organ failure. However, there are not enough allografts available to close the gap between supply and demand for organ transplantation.[459] Therefore, alternative solutions such as xenotransplantation remain of great interest. Xenotransplantation has been defined by the world health organization (WHO) as "*any procedure that involves the transplantation, implantation or infusion into a human recipient of either: live cells, tissues, or organs from a non-human animal source; or human body fluids, cells, tissues or organs that have had ex vivo contact with live non-human animal cells, tissues or organs*".[460] In 1667 xenotransplantation was mentioned for the very first time; back then they investigated xenotransfusion of blood from lambs into human beings.[460, 461] In Vienna, at the medical university of Vienna in January 1902 the surgeon Emerich Ullmann reported the first successful renal autotransplantation implanted in the neck of a dog. Soon thereafter he presented the first xenotransplantation by implanting a dog's kidney into a goat. Since his work immediately had great impact on the medical world he attempted to transplant a pig's kidney into a young uraemic woman. However, he failed and stopped his research on this field, but his ideas survived until now.[462] Especially pigs as a source of xenotransplantation remained of great interest over the years, due to the short maturation time, the large litter size and other physiological similarities to humans. Therefore, research work on genetic engineering remains ongoing; to develop and improve strategies to make porcine organs resistant against rejection.[463] Nevertheless, genetic engineering techniques are not sufficient enough to diminish all genetic discrepancies between pigs and humans resulting in immunological rejections. Immunological rejection to discordant xenotransplantation can be divided into 3 main types: Hyperacute xenograft rejection (HAR), acute humoral xenograft rejection (AHXR), and acute cellular rejection (ACR).[464]

5.2.1 Hyperacute Rejection and Acute Humoral Xenograft Rejection

Hyperacute rejection (HAR) is known as immediate graft destruction occurring within minutes to hours after transplantation of a wild-type porcine organ into a human being.[465] In this acute situation HAR can be treated by depleting anti-pig antibodies or by inhibiting the complement system via plasmapheresis.[466] However, this treatment ideas can only prolong graft survival for 24h or even a week, since antibodies are able to recover, leading to delayed xenograft rejection (AHXR).[467] Preformed naturally pre-existing antibodies of the recipient mediate HAR, which is known as a type of humoral rejection. These preformed antibodies bind to xenoantigenic epitopes of pig endothelial cells that further activates the complement system. Complement activation induces endothelial cell lysis to destroy the graft vasculature and subsequently leading to xenograft failure.[468] The main xenoantigen causing HAR is the already well described alpha- Gal epitope.[469] Since kidneys and hearts from alpha- Gal knockout pigs transplanted into non-human primates were also rejected over several days, further evidence came up that non-Gal xenoantigens seem to exist. These non-Gal antigens present another drawback for xenotransplantation of grafts from alpha-Gal knockout pigs to humans. [460, 470, 471] There are two main non-Gal epitopes: *N*- glycolylneuraminic acid (Neu5Gc) and the SDa blood group.[472, 473]

5.2.2 Acute cellular Rejection

Acute cellular rejection (ACR) occurs within days to weeks after xenotransplantation and can be mediated by both arms: the innate and the adaptive immunity. Several cell types can carry out an ACR including macrophages, NK cells, neutrophils, dendritic cells, T cells, and B cells.[474] Itescu and colleagues reported already in 1998 of this secondary immunogenic response after HAR characterized by xenograft inflation with NK cells and macrophages in pig to primate cardiac xenotransplantation in baboons. NKs cells mediated lysis of porcine endothelium.[475]

5.3 Meat allergy, the Alpha-Gal syndrome

The oligosaccharide, alpha-Gal is present in mammalian food as both glycolipids and glycoproteins, including meat, internal organs (such as kidney or tripe), milk and other dairy, and gelatin, but also other products such as the monoclonal antibody cetuximab and the zoster vaccine.[476-478]The alpha-Gal syndrome is characterized by a delayed occurrence of symptoms after meat consumption; typically 3–6 hours after a relevant exposure. [479] The most plausible explanation involves the time required for processing, digestion and transit of alpha-Gal epitopes to target tissues. Thereby alpha-Gal linked glycolipids of mammalian cells and tissues revealed further interest due to the slow kinetics of the lipid metabolism.[480, 481] The slow kinetics of the lipid metabolism seems particularly important for the delayed allergic response. Indeed, this hypothesis was emphasized by other observations that found that lean meat, particularly venison, is less likely to trigger reactions in alpha-Gal allergic subjects than fatty cuts. [482]

Platts Mills described the idea of alpha-gal-specific IgE formation in the context of meat allergy for the very first time.[483] Soon thereafter, Kollmann et al. found that there is a link between meat allergy and elevated alpha-Gal specific IgG, IgG1, and IgG3 serum concentrations.[484] IgE sensitization to alpha-Gal is known as related to tick bites. In the United States there is evidence that the lone star tick is a dominant 'vector' for inducing IgE to alpha-Gal, a finding that is supported by the strong overlap between patients diagnosed with the alpha-Gal syndrome and the established distribution of the lone star tick.[485] IgE sensitization to alpha-Gal marked regional variability and mainly occurs in areas of the southeast, lower Midwest and coastal Atlantic. The population prevalence of IgE sensitization to alpha-Gal is substantially greater than the prevalence of alpha-Gal syndrome that is diagnosed clinically. Even though high titers to alpha-Gal correlate with the risk of clinical symptoms, there are subjects with high titers of IgE to alpha-Gal, without developing symptoms after digesting meat.[485, 486] A recent report on high-risk forest workers found that 58 of 300 subjects were sensitized with IgE to α-Gal. However, only 5 of these cases had symptoms consistent with alpha-Gal syndrome; with other words: 90% of the sensitized subjects in the cohort did not report relevant symptoms.[487]Furthermore, another study reported a link between IgE sensitization to alpha-Gal and coronary artery disease (CAD): In 118 patients with CAD undergoing coronary catheterization the amount of atherosclerotic plaques were significantly greater in patients with detectable titers of IgE to alpha-Gal. This finding was most striking in the relatively younger subjects (younger than 65 years). In these "younger" subjects plaques had greater calcification and fibrofatty and necrotic content. [486, 488] Besides, Mangold and

colleagues reported in 2011 that alpha-Gal–specific antibodies remain stable in healthy volunteers.[489]

5.4 Alpha-Gal and bioprosthetic heart valves

Bioprosthetic heart valves (BHV) cross-linked with glutaraldehyde are the most common implanted bioprostheses for cardiac surgery.[490] However, glutaraldehyde cannot efficiently cross-link carbohydrates due to the absence of amino groups and therefore cannot mask alpha-Gal epitopes.[490] In addition, various decellularization procedures are applied to eliminate porcine cells and create a cell-free matrix. However, decellularization protocols vary greatly in their efficiency of cell removal, and incomplete decellularization remains associated with the presence of alpha-Gal epitopes, which trigger, among two other main antigens,Neu5Gc and Sda and many others, immune reactions against BHVs.[491, 492]

At our institution, in 2003 the first tissue engineered decellularized porcine heart valve were implanted into four children between two and eleven years old. Even though early postoperative recovery was uneventful, three children died subsequently. Two children died after 6 weeks and 1 year after implantation with severely degenerated heart valves. The third child died on the 7th postoperative day, because of valve rupture. The fourth child survived due to prophylactical graft explanation 2 days after implantation. After explanation, All four BHVs showed macroscopically severe inflammation, structural failure and severe degeneration of the leaflets.[493] In addition, decellularization of these four BHVs induced substantial loss in valve stiffness, leading to significant extracellular matrix disruption.[490, 494] At this point it was clear that decellularized matrix couldn't be biologically inert. Solely decellularized porcine heart valve matrix were highly platelet activating. Therefore the same study group brought further evidence that seeding the matrix with endothelial cells abolish platelet activation. This is especially important given that platelets are known to be involved in pro-inflammatory processes beside their thromogenetic features.[495] In 2005 Koncaki and colleagues reported for the first time an on-going specific immune response against alpha-Gal residues on the valve matrix of newly implanted commercially available BHVs, resulting in a significant raise of alpha-Gal specific IgM ABs in patients' sera 10 days after cardiac surgery.[30] In 2009, the same study group analysed patient's sera 3 months after BHV implantation and found a similar increase of alpha-Gal specific IgG ABs, indicating a common AB class switch induced by continuous antigen exposure.[31, 496] In detail, the IgG3 subclass was mainly induced against the alpha-Gal

epitopes. IgG3 is the IgG subclass which is capable to activate complement, and the most effective subclass in inducing cytolysis.[31, 497]

Recently, published in Nature medicine in 2022, Senage and co-workers confirmed in a larger multicentre study on 1668 patients that BHVs are immunogenic and elicit an concomitant raise of anti-alpha-Gal and anti-Neu5Gc IgG serum concentrations within the first 6 months after BHV implantation. Furthermore, they found a non-significant raise of these antibodies in the control group. The control group consisted out of patients who received either a mechanical heart valve or underwent CABG surgery. The authors concluded that ECC procedures may contribute to an antibody increase without novel exposure to antigens. During follow-up after BHV implantation anti-alpha-Gal, as well as anti-Neu5Gc antibodies increased. However, anti-alpha-Gal IgG levels decreased more rapidly, while anti-Neu5Gc IgG levels remained elevated for a longer period of time. The study group concluded that there must be a longer exposure of implanted valves to anti-Neu5Gc IgG than to anti-alpha-Gal IgG antibodies.[498] A different study reported that after implantation of glutaraldehyde-fixed BHVs patients exhibited reactivity toward 19 graft proteins. Some of them were homologous to human proteins responsible for autoantibody formation in various human diseases.[499] Another research work found that patients with implanted BHVs display increased titers of serum antibodies against porcine albumin and type IV collagen.[500]

The immune reactivity of implanted commercially available BHVs is known to have an age dependent effect. While in patients older than 70 years, valves may have a durability of up 10-20 years; BHVs fail in up to 100% within 5 years of all patients younger than 35 years old.[490, 501, 502] The reduced durability of BHV especially in young recipients results out of extensive antibody responses in the young against xenogenic antigens, inducing chronic inflammation, calcification, pannus formation or perforation of the valve leaflets of the BHV. Therefore, according to guidelines younger patients should mainly receive mechanical heart valves, which require lifelong anticoagulation therapy as a drawback.[490, 503, 504]

5.5 Transcatheter aortic valve replacement

In the early 1960y Davies H. described for the first time a catheter- mounted valve for the treatment of aortic insufficiency in an animal model.[505] Bonhoeffer and co-workers replaced the pulmonary valve with a transcatheter implantation technique in humans for the very first time.[506] In 2002 Cribier performed the first successful transcatheter aortic valve replacement (TAVR). He performed an antegrad transseptal catheterization to insert

a newly developed percutaneous heart valve in a patient with calcific aortic stenosis (AS) who was considered inoberable.[507] The valve was implanted antegrade via a femoral transvenous access. The guidewire was advanced across the atrial septum and then dilated for the crimped aortic valve prosthesis. Subsequently, the prosthesis was implanted in midposition of the aortic valve.[507] The antegrade transseptal approach had several limitations, due to the technical complexity and associated risk factors including the development of acute mitral regurgitation by the guidewire loop inside the left ventricle with subsequent hemodynamic deterioration.[507]

Soon thereafter, the retrograde transfemoral arterial approach was implemented using rapid ventricular pacing to minimize pulsatile transaortic flow. This approach is now known as the access of choice.[508] Nevertheless, several groups investigated a second route for TAVR, namely the transapical approach to the aortic valve. For this approach a left mini-thoracotomy has to be performed to get access to the aortic valve through the left ventricular apex. This technique emerged from the experience with patients with severe peripheral arterial disease, which is an absolute contraindication for the transfemoral arterial approach.[509] These two approaches are now co-existing techniques of daily clinical praxis. [505] The transfemoral approach is known as the access of choice, with a decreased periprocedural risk. However, the size of the iliofemoral circulation is important to determine prior to use the transfemoral access. Hybrid operating rooms are beneficial for high-risk transfemoral TAVR owing to larger space, cardiopulmonary bypass backup, and the opportunity for prompt conversion to open surgery for complications such as valve migration, myocardial perforation, and vascular injuries.[510] Short- and long-term results of both implantation modalities are improving with increasing experience and constant technological innovations as valve and delivery systems are evolving rapidly.[511]

5.5.1 Aortic valve stenosis

AS is the third leading cardiovascular disease after hypertension and coronary artery disease and the most common valve disease in the western population with an estimated overall prevalence of up to 3% in patients older than 75 years. [512] The reported prevalence of AS among patients aged 50-59 years is only about 0.2% and is rising to almost 10% in patients aged over 80 years.[513]AS results out of an inflammatory process induced by endothelial damage due to mechanical stress; lipid penetration leading to fibrosis, leaflet thickening, and finally calcification. [514] Calcific AS causes increased leaflet stiffness and narrowing of the aortic valve orifice, resulting in an increased pressure gradient across the valve. [515] AS presents a prolonged subclinical period, defined as aortic sclerosis. During

this period the calcific process is already on-going on the aortic valve. However there is still no transvalvular gradient present. Progressive calcification results in further aortic valve narrowing with concomitant left ventricular pressure overload and resultant left ventricular hypertrophy, thereby leading to the classic triad of AS symptoms: heart failure, syncope and angina.[514] Untreated symptomatic AS is rapidly fatal with an annual mortality rate of 25% and an average survival of 2 to 3 years.[516]

5.5.2 Diagnosis

The severity of AS can be assessed noninvasively by Doppler echocardiography according to following criteria:

- maximum aortic jet velocity >4.0 m/s
- mean transvalvular pressure gradient >40 mmHg
- or continuity equation valve area <1.0 cm^2
- or valve area indexed for body surface area <0.6 cm^2

Invasive diagnostic evaluation of AS via cardiac catherization is only indicated if there are discrepancies between the clinical evaluation and the echocardiogramm.[516, 517]

5.5.3 Therapeutic options of Aortic valve stenosis- ESC/EACTS Guidelines of 2021

To treat AS two different treatment options exist: surgical aortic valve replacement (SAVR) and TAVR.[517] According to the ESC/EACTS guidelines of 2021 intervention is recommended in patients with clinical symptoms of AS, in addition to echocardiographic findings comprising high-gradient AS with a mean gradient ≥ 40 mmHg, a peak velocity ≥ 4.0 m/s and a valve area ≤ 1.0 cm^2 or ≤ 0.6 cm^2/ m^2.[518] Intervention is also recommended in patients showing clinical symptoms with severe low-flow, low-gradient AS with a stroke volume index ≤ 35 mL/m^2, a mean gradient ≤ 40 mmHg, a reduced ejection fraction of < 50% and evidence of flow reserve (1B recommendation).[519] Furthermore, if patients are asymptomatic with severe AS with a reduced systolic LV dysfunction <50% without another cause for reduced EF, intervention is recommended (1B recommendation).[520]
Guidelines recommend performing aortic valve interventions only in specialized Heart Valve Centers with a multidisciplinary Heart Team Approach that declare their local expertise and have active interventional cardiologic and cardiac surgical programs (1C recommendation). The multidisciplinary Heart Team has to make the choice between

SAVR and TAVR after evaluation of anatomical, clinical and procedural factors. The treatment choice should be further discussed with each individual patient (1C recommendation).[521] According to the guidelines SAVR is recommended in younger patients below 75 years who have a low surgical risk with a Society of Thoracic Surgery-Predicted Risk Of Mortality (STS- PROM)/European System for Cardiac Operative Risk Evaluation (EuroSCORE) II < 4% or in patients who are operable and unsuitable for minimal invasive transfemoral TAVR (1B recommendation).[522] In contrast, TAVR is recommended in patients older than 75 years, in patients, who have a high surgical risk with a STS- PROM/EuroSCORE II >8% or in patients who are unsuitable for surgery (1A recommendation).[523] In patients who were considered as inoperable and unsuitable for transfemoral TAVR non-transfemoral TAVR can be performed (IIB recommendation).
In severe AS patients who undergo another heart surgery SVAR is recommended (1C recommendation). In patients with moderate AS undergoing another surgical procedure SAVR should be discussed in the Heart Team (2a C recommendation).[521]

Balloon Valvuloplasty

Balloon valvuloplasty is used for palliation in patients in whom aortic valve replacement cannot be performed due to comorbidities.[516] Balloon valvuloplasty can applied as a bridge to SAVR or TAVR.[525] However, the hemodynamic benefit of this therapy remains transient, lasting only for 6 months.[526]

Surgical Aortic Valve Replacement

Surgical aortic valve replacement (SAVR) is performed with either mechanical or bioprosthetic aortic valves. Biological prosthetic heart valves are either bovine or porcine. They do not require prolonged anticoagulation. However, it remains unclear if their life span is comparable with mechanical heart valves.[517] One study comparing bioprosthetic versus mechanical heart valves in patients aged 50-69 years demonstrated that patients with mechanical heart valves had better survival rates. In this study the risk for aortic valve reoperation was higher, however major bleeding was lower in patients who received a BHV.[527] In contrast, another study with a similar age group comparing bioprosthetic versus mechanical heart valves demonstrated no significant difference in 15-year survival or stroke rate. [528]

Transcatheter aortic valve replacement

TAVR is discussed above.

5.6 SAVR Versus TAVR to treat aortic stenosis

The advantage of TAVR for inoperable or high-risk surgical patients was analyzed in several studies.[529, 530] The PARTNER I trial investigated in 2010 358 patients with AS who were not considered to be suitable for surgery in 21 centers. At 1 year, all cause mortality was 30.7% with TAVR and 50.7% with standard therapy. Among survivors at 1 year, the rate of cardiac symptoms was lower in TAVR patients. However, 30 days after intervention TAVR was related to a higher incidence of major strokes (5.0% vs. 1.1%) and major vascular complications (16.2% vs. 1.1%). One year after TAVR there was no deterioration in the functioning of the BHV, as assessed via echocardiography.[531] Investigating 5-year outcome TAVR reduced all-cause mortality by 21.8% compared to standard therapy. The number needed to treat to save 1 life was 5. In high-risk but operable patients the 5-year mortality for TAVR and SAVR was equivalent.[529] In 2014 TAVR versus SAVR was investigated in the so-called US CoreValve trial. In this study 795 patients with severe AS and an increased surgical risk were included.[532] At 3 years, all-cause mortality was lower in the TAVR group compared with SAVR (32.9% vs 39.1%).[532, 533] In 2016 the PARTNER 2 trial analyzed 2032 patients with an intermediate surgical risk with severe AS who were randomly assigned to either TAVR or SAVR.[534] The all cause mortality and the rate of disabling strokes was similar in the TAVR and the SAVR group. After 2 years 19.3% and 21.1% TAVR and SAVR patients died from any cause. In a sub-analysis on patients undergoing TAVR transfemorally, TAVR resulted in a lower rate of death or disabling stroke than SAVR. In contrast, in patients undergoing TAVR transapically, there was no difference between TAVR and SAVR. Furthermore, fewer TAVR patients developed acute kidney injury, severe bleeding, and new-onset atrial fibrillation, but fewer SAVR patients had major vascular complications and paravalvular aortic valve regurgitation.[532] In 2015 in North Europe the NOTION randomized trial compared TAVR with SAVR patients. The mean age of all patients was 79 years and 82% were considered to have a low surgical risk. They did not find statistically significant differences between TAVR and SAVR with regard to all-cause mortality, stroke and myocardial infarction.[535] The PARTNER 3 trial, published in 2019 randomized 1000 patients diagnosed with severe AS who had a low surgical risk to undergo either TAVR or SAVR. The mean age of the cohort was 73 years. In this trial the mortality rate after one year was significantly lower in the TAVR group compared to SAVR. Additionally, 30 days after intervention/surgery TAVR patients had a significantly lower rate of strokes, deaths and new-onset atrial fibrillation. Patients after TAVR also had a shorter duration of hospitalization.[524] Two-year outcome of the PARTNER 3 trial continue to show a

numerical benefit of TAVR over SAVR with regard to the study's primary endpoint of death, stroke, or cardiovascular rehospitalization.[536]

5.7 Transcatheter edge-to-edge repair - MitraClip®

The transcatheter edge-to-edge repair (TEER) with MitraClip® was introduced for the very fist time as a therapeutic option in humans in 2003.[537] The idea of TEER was based on the Alfieri stich, introduced in 1995 as a treatment choice for high-risk patients in who prolonged CPB should be avoided. The Alfieri stich can be performed very quickly through the aortic valve. [538] TEER is now an accepted alternative procedure for high risk surgical patients with secondary mitral regurgitation (MR).[539] Recently, the efficacy and safety of TEER has been assessed in two large randomized controlled trials, the MITRA-FR and the COAPT trial. Even though, these two trials analyzed the same patient cohort, results of these two trials were diametrically opposed: The MITRA-FR found a neutral result, while the COAPT favoured TEER in contrast to medical treatment only.[540, 541] The ESC/EACTS guidelines from 2021 recommended on the basis of both trials to perform TEER only in patients with severe systolic MR who remain symptomatic despite goal directed medical therapy. This decision has to be made by a structured collaborative Heart Team (Class I, Level B). In contrast, if patients require revascularization or another heart surgical therapy, valve surgery is recommended (Class I, Level B). In symptomatic patients, who were judged inoperable by the Heart Team TEER should be considered (Class IIa, Level C). [542] Technical developments and growing experience with TEER decreased the major adverse event rate from 15% in 2005 to 3.5% in 2020, taking into account that even more complex lesions have been treated more recently.[543, 544]

5.7.1 Indications for TEER Mitral regurgitation

MR is defined as reversal blood flow from the left ventricle back into the left atrium during systole. Therefore MR leads to an increased blood volume within the left atrium and hence an increased preload delivered to the left ventricle during diastole that induces left ventricular volume overload and increases stroke volume. As a consequence, ventricular remodeling has to occur to maintain cardiac output. In some patients with MR, initially even an increase in ejection fraction can be observed. However, according to the regugitant fraction, effective ejection fraction can still be reduced. Over time, this positive feedback loop causes ventricular and mitral annulus dilatation, and reduced coaptation of leaflets leading to progressive decline of MR. Subsequently, if volume overload further progresses, excitation-contraction coupling becomes impaired leading to further dilatation of the left ventricle and reduces contractility, resulting in a reduction of ejection fraction.[545] MR can occur due to two different etiologies comprising primary and secondary MR.

Primary MR also known as structural, degenerative or organic MR develops because of myxomatous degenerative disease of the mitral valve including rheumatic heart disease, fibroelastic deficiency, Barlow disease or endocarditis. In contrast, in patients with secondary of functional MR, the valve apparatus is anatomically intact and the reversal blood flow results from a disproportion between closing and tethering forces on the valve secondary to left ventricular dilation and dysfunction. It occurs most frequently due to dilated or ischemic cardiomyopathy.[546, 547]

5.7.2 Diagnosis

Initially mild functional MR occurs without presentation of clinical symptoms. When the patient notices symptoms for the first time severe MR is often already present. These symptoms include: dyspnoea, fatigue, heart palpitations and swollen feet and ankles.[548]

The Carpenter's classification categorize patients with MR according to leaflet motion during echocardiography as depicted below:

- **Type I:** normal leaflet motion with annular dilation or leaflet perforation with a central regurgitation jet
- **Type II:** excessive leaflet motion, due to papillary muscle or chordal rupture, redundant chordae and an eccentric regurgitation jet
- **Type III:** restricted leaflet motion
    - IIIa: restricted leaflet motion during systole and diastole with a central or eccentric jet
    - IIIb: restricted leaflet motion during diastole, due to papillary muscle dysfunction with left ventricular dilation and either a central or an eccentrical jet.[549, 550]

Severity of MR can be assessed using heart echocardiography as depicted in **Table 6.**

Table 6: Echocardiographic parameters of MR

Adopted from Otto et al. [548]

Echocardiographic parameters	Mild 1+	Moderate 2+	Moderate to severe 3+	Severe 4+
Ejection fraction; %	≥ 50	35 - 50	20 - 35	≤ 20
Effective regurgitation orifice area; mm^2	≤ 20	20 - 29	30 - 39	≥ 40
Regurgitant volume; ml/beat	≤ 30	30 - 44	45 - 59	≥ 60
Regurgitation fraction;%	≤ 30	30 - 39	40 - 49	≥ 50
Jet area/ Left atrial area; %	≤ 10	10 - 20	20 - 45	≥ 45

According to the American Heart Association/American College of Cardiology (AHA/ACC), there are 4 stages of functional MR:

⇒ **Stage A**- patients are at risk for functional MR. These patients are diagnosed with coronary artery heart disease or non-ischemic dilated cardiomyopathy without having symptoms related to functional MR.

⇒ **Stage B**- patients have mild to moderate mitral regurgitation in echocardiography but do not present any symptoms of heart failure.

⇒ **Stage C**- patients are diagnosed echocardiographically with severe MR, received medical treatment but do not present any symptoms.

⇒ **Stage D**- patients are diagnosed with severe mitral regurgitation in echocardiography and have symptoms such as reduced exercise tolerance, dyspnea and fatigue during exercise. Medical therapy such as cardiac resynchronization and coronary revascularization does not realease symptoms. [548]

5.7.3 Treatment of MR

Therapeutic strategies for MR can be subdivided into medical, surgical and interventional treatment. Treatment choice is dependent on disease severity, chronicity, patients' comorbidities and MR etiology. Details are discussed below.

Medical therapy

According to literature medical treatment with Angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II receptor blockers (ARB) are known to delay disease progression in patients with asymptomatic severe MR. However, there is no evidence that medical treatment reduce regurgitant volume and left-ventricular dilatation in patients with chronic primary MR.[551] Moreover, medical treatment with ACEI/ARBs even increased MR severity in patients with hypertrophic cardiomyopathy and in patients with mitral valve prolapse.[552] In contrast in patients with secondary MR, but not primary MR Beta-blockers have shown a survival benefit for patients.[553]

Surgical therapy

Two different surgical approaches exist to treat MR: Valve repair and valve replacement. According to the ACC/AHA guidelines mitral valve repair has to be preferred over mitral valve replacement due to reduced MR recurrence.[555] However, in patients with severe tissue destruction mitral valve replacement may is favorable over valve repair.[556] For mitral valve replacement mechanical prostheses are preferable over biological heart valves due to increased durability in younger patients.[539] Surgery is recommended in patients with chordal or papillary muscle rupture and in patients with infective endocarditis. In contrast, patients with secondary MR due to ischemia may require coronary revascularization instead of a valvular procedure. Patients with acute symptomatic MR and/or patients with an effective regurgitant orifice of more than 40 mm^2, measured via echocardiography, require a surgical procedure.[545] Moreover, MR surgery is indicated in patients with reduced left ventricular function or in patients with left ventricular dilatation with an end-systolic diameter of more than 4.5 cm.[557] Additionally, guidelines recommend performing surgery in symptomatic or asymptomatic patients with primary severe MR with an ejection fraction over 30% or between 30% to 60%.[558]

Interventional therapy

Interventional therapy is described above.

5.8 NETs, the complement system and sST2

5.8.1 Neutrophils, NETs and CitH3

Neutrophils play an essential role for the initial defense against entering pathogens. The production of neutrophils takes place in the bone marrow; these cells are subsequently released into the blood- stream ready to be recalled to the site of infection. Neutrophils are the most abundant granulocytes of human beings accounting for up to 40 to 70% of all white blood cells.[559] These cells perform their anti-microbial effects via phagocytosis, degranulation and neutrophil extracellular trap (NET)osis. NETosis is a process in which extracellular web-like structures were released, termed NETs. NETs consist out of decondensed chromatin filaments that are coated in histones and antimicrobial proteins.[560, 561] Two different pathways of NEtosis exist: The "lytic/suicidal" pathway also known as the *slow cell death pathway* and the "vital" pathway, further termed as the *alternative pathway*.[562] The "lytic/suicidal" pathway can be stimulated by Phorbol 12-myristate 13-acetate (PMA), a plant-derived natural organic compound, antibodies, cholesterol crystals, bacterial lipopolysaccharide (LPS) and ILs.[563] During the "lytic/suicidal" pathway via the Raf/MEK/ERK signal transduction cascade calcium has to be released from the endoplasmic reticulum to enhance phosphorylation of NADPH oxidase subunits.[564] As a next step, the neutrophil elastase has to translocate to the nucleus to decondensate nuclear chromatin and activate peptidyl arginine deiminase 4, a calcium- dependent enzyme that citrullinates histones, especially H3 (citH3).[565] Thereby, the membrane of the nucleus breaks down and loses his specific lobular shape. Subsequently, decondensed chromatin comes out of the nucleus into the cytosol.[566] Furthermore, the plasma membrane dissolves and decondensed chromatin together with proteins and histones are released as reticular structures outside the lytic neutrophil cell.[567]

As already discussed elsewhere, Gal knockout pigs were considered promising for xenotransplantation. Yoo and colleagues reported in 2016 that NETs, mainly composed out of DNA–histone complex were not only induced by wild type porcine endothelial cells (pEC), but also in pECs of Gal knockout pigs. The authors concluded that thrombotic dysregulation induced by NETs still remain an obstacle in xenotransplantation of Gal knockout pigs. The authors added, that further coagulation strategies seem required in xenotransplantation to block soluble histones.[568]

5.8.2 Complement factor 3a

The complement system has an important role during xenotransplantation, with three different pathways: the classical, the alternative and the lectin pathway.[569] *The classical pathway* plays a major role during HAR by destroying a wild-type xenograft immediately after transplantation into a human being.[570] Antibody binding to xenoantigens like gal activates the *classical pathway*; the anti-antibody-antigen complex triggers C1q, activates C1r, C1s, and then cleaves C4 and C2 to form C4b2a.[570] In contrast, the *alternative pathway* is only activated occasionally, without activation via antigen-antibody bindings.[571] The *lectin pathway* was the last discovered pathways, therefore extact details of the mechanism remain incomplete.[572] All three pathway end up in a so called “common pathway”, in which C5 convertase cleaves C5 into C5a and C5b, C5b interacts with C6-C9 to form the membrane attack complex (C5b-9) which induces lysis, damage and activation of target cells.[570, 573]

5.8.3 ST2

ST2 is a protein of the interleukin-1 receptor family. Alternative splicing of the ST2 gene generates three mRNAs, corresponding to a longer membrane- anchored form (ST2L), a shorter released form (soluble ST2), and a membrane bound variant form (ST2V).[82] Activation of the membrane-bound ST2-receptor via IL-33 activates NFκB and ultimately, the expression of T-helper cell (Th2) associated cytokines comprising IL-4, IL-5 and IL-13 and enhances the pro-inflammatory cytokine production of IL-8 and IL-6.[83] Therefore a potential role for IL-33 may be to alert the immune system of cell or tissue damage, as an endogenous ‘danger’ signal or ‘alarmin’ after the release from dead or dying cells during trauma or infection as described by Moussion.[84] sST2 acts as a so-called *decoy receptor* for IL-33 by capturing IL-33 to reduce Th2 inflammatory responses.[86] sST2 interacts with macrophages and suppresses pro-inflammatory proteins by down-regulation of NFκB. [87, 88] Mildner et al. found that human myocytes and pneumoepithelial cells are the main sources of circulatory bioactive sST2 and that endotoxin triggering, via induction of inflammatory cytokines in PBMC, augment sST2 secretion in alveolar epithelial cells and cardiac myocytes.[85] The authors assumed that lung cells may require sST2 to ensure endotoxin tolerance, since the lungs have an enormous surface, which is exposed to endotoxins.[88] Furthermore, a massively enhanced sST2 secretion has been reported after heart surgery.[87] Besides, sST2 was identified as a biomarker for cardiac heart failure.[89] Recently, in 2021 Lingitz and colleagues investigated the impact of an infection with

Coronavirus Disease 2019 on sST2 secretion. The study group found that immune suppressive sST2 cytokine serum concentrations were massively enhanced in patients with COVID-19. Further, sST2 levels were associated with mortality, invasive ventilation, and oxygen support. The authors concluded that Coronavirus Disease 2019 may be more likely explained by immunodeficiency than by hyper-inflammation.[90] Another study investigated circulating IL-33 and sST2 in each trimester of normal pregnancy and in women with pre-eclampsia. While IL-33 did not change throughout normal pregnancy, or between non-pregnant, normal pregnant or pre-eclamptic women, sST2 was significantly increased in the third trimester of normal pregnancy and was further increased in pre-eclampsia. This increase in sST2 cytokine serum concentrations was even seen before disease onset.[91]

5.9 Aims and Hypotheses

The aim of this observational cohort study was to analyze whether BHVs implanted via TAVR induce an alpha-Gal–specific humoral immune activation of total IgG, IgG subclasses, and IgE, enhance the complement system via C3a, induce NETosis determined via CitH3 and activate the IL-33/ST2 pathway within 90 days after the procedure. Baseline serum concentrations, determined prior to intervention and patients undergoing the MitraClip intervention served as a control.

5.9.1 Hypothesis No. 1:

Null hypothesis H0: There is no difference in serum concentrations of total IgG and IgG subclasses, citH3, sST2, IL-33 and C3a between baseline and 3 Months after the TAVI procedure.

Alternative hypothesis H1: There is a difference in serum concentrations of total IgG and IgG subclasses between baseline and 3 Months after the TAVI procedure.

5.9.2 Hypothesis No. 2:

Null hypothesis H0: There is no difference in serum concentrations of total IgG and IgG subclasses, citH3, sST2, IL-33 and C3a between baseline and 3 months after the MitraClip procedure.

Alternative hypothesis H1: There is a difference in serum concentrations of total IgG and IgG subclasses, citH3, sST2, IL-33 and C3a between baseline and 3 months after the MitraClip procedure.

CHAPTER SEVEN: RESULTS

6.1 Prologue

Alpha-Gal carrying porcine or bovine BHVs are commonly used biological non-degradable scaffolds applied for SAVR. Though the association between alpha-Gal carrying heart valves and systemic immune activations, calcification and degradation of these valves has also ready been topic of several research works. However, it remains undiscovered whether BHVs implanted for TAVR carry the same epitope, thereby inducing an immune reaction. We therefore investigated in 27 patients whether TAVR cause an alpha-Gal specific antibody dependent or independent immune activation 3 months after the procedure. Patients undergoing TEER with MitraCLIP served as a control group. We analysed alpha-Gal specific IgG, IgG1, IgG3, IgE concentrations AND sST2, C3a and citH3 levels prior to intervention and 3 months thereafter.

A copy of the original article, published in THE JOURNAL JTCVS open (open source) is depicted below. Author contributions are declared on page II.

Inflammatory immune response in recipients of transcatheter aortic valves

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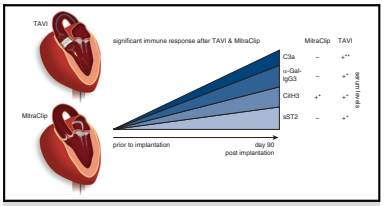
ABSTRACT

Objective: Transcatheter aortic valve implantation (TAVI) is rapidly replacing cardiac surgery due to its minimal invasiveness and practicality. Midterm immunological studies on the biocompatibility of galactose-alpha-1,3-galactose (α-Gal)–carrying bioprosthetic heart valves for TAVI are not available. In this study we investigated whether bioprosthetic heart valves employed for TAVI augment an α-Gal–specific antibody-dependent and antibody-independent immune response 3 months after TAVI implantation.

Methods: This prospective observational study included 27 patients with severe aortic valve stenosis undergoing TAVI and 10 patients with severe mitral valve regurgitation treated with a transcatheter MitraClip (Abbott Laboratories, Abbott Park, Ill) procedure. Blood samples were drawn before and 90 days after treatment at a routine checkup. Serum samples were analyzed using enzyme-linked immunosorbent assay. Serum concentrations of α-Gal–specific immunoglobulin (Ig) G, IgG subclasses and IgE, complement factor 3a, NETosis-specific citrullinated H3, and the systemic inflammation markers soluble suppression of tumorigenicity and interleukin 33 were evaluated.

Results: Three months after TAVI, we found significantly increased serum concentrations of α-Gal–specific IgG3, complement factor complement factor 3a, citrullinated H3 levels, and soluble suppression of tumorigenicity ($P = .002$, $P = .001$, $P = .025$, and $P = .039$, respectively). Sensitization of α-Gal–specific IgE antibodies occurred in 55% of all patients after TAVI.

Conclusions: Our results indicate that TAVI elicits a midterm, specific humoral immune response against α-Gal and causes an unspecific humoral inflammation compared with patients undergoing MitraClip implantation. This observation will lead to a better understanding of postintervention morbidity and the long-term durability of bioprostheses and indicates that caution is appropriate when designing implantation strategies for younger patients. (JTCVS Open 2021;6:85-96)



Significant immune response after transcatheter aortic valve implantation (TAVI) and MitraClip (Abbott Laboratories, Abbott Park, Ill). Three months after TAVI we found significantly increased serum concentrations of galactose-alpha-1,3-galactose (α-Gal-specific IgG3), complement factor 3a (C3a), citrullinated H3 (citH3), and soluble suppression of tumorigenicity (sST2) levels compared with baseline levels ($P = .002$, $P = .001$, $P = .025$, and $P = .039$). Three months after MitraClip implant, citH3 levels were significantly elevated compared with baseline levels ($P = .03$).

CENTRAL MESSAGE

Transcatheter aortic valve implantation induces an α-Gal-specific humoral immune response and augments the complement activities NETosis and ST2 within 90 days of valve implantation.

PERSPECTIVE

Next-generation transcatheter aortic valve implantation, deficient for Gal, will show whether immune activity can be dampened with the potential consequence of increased valve durability.

See Commentaries on pages 97 and 99.

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Abbreviations and Acronyms

α-Gal	= galactose-alpha-1,3-galactose
BHV	= bioprosthetic heart valve
C3a	= complement factor 3a
CitH3	= citrullinated H3
DAPI	= 4′,6-diamidino-2-phenylindole
ELISA	= enzyme-linked immunosorbent assay
Ig	= immunoglobulin
IL	= interleukin
mAb	= monoclonal antibody
NET	= neutrophil extracellular traps
NT-proBNP	= N-terminal pro-brain natriuretic peptide
sST2	= soluble suppression of tumorigenicity-2
TAVI	= transcatheter aortic valve implantation

▶ Video clip is available online.

Transcatheter aortic valve implantations (TAVI) performed by cardiologists and cardiac surgeons will outnumber conventional cardiac operations in the future due to their minimal invasiveness, practicality, and the aging population.[1-3] However, midterm immunological studies on the biocompatibility of galactose-alpha-1,3-galactose (α-Gal)–carrying bioprosthetic heart valves (BHV) for TAVI have not been performed so far.

The α-Gal epitope is widely accepted as the major elicitor in the pathogenesis of immune activation after xenotransplantation and implantation of glutaraldehyde-fixed BHVs. The relationship between tissue α-Gal–specific immune reactivity and dystrophic calcification, inflammation, and leaflet tearing in bioprostheses in vivo is widely accepted.[4-7]

Beside BHV-induced α-Gal–specific antibody-dependent humoral immune responses, xenografts activate the classical pathway of the complement system when antibodies bind to antigens such as α-Gal on their surfaces, and thereby trigger C1q to activate C1r and C2s, cleave C4 and C2 and form C4b2a (C3 convertase), and activate complement factor 3a (C3a).[8,9]

Most recently, another deleterious immune activation process in xenotransplantation has gained prominence, namely the ejection of DNA-histone complexes into the extracellular space from activated neutrophils to form neutrophil extracellular traps (NETs).[10,11] Increased NET formation is well known in various clinical conditions, including sepsis, trauma, autoimmune diseases, deep vein thrombosis, atherosclerosis, and thrombotic microangiopathy.[12,13]

Because multiple nosologies share humoral and cellular activation pathways, we asked whether TAVI increases circulating soluble suppression of tumorigenicity-2 (sST2) and its counterpart interleukin (IL)-33, a known biological alarmin that would serve as an additional biological marker for ongoing inflammatory processes.[14-16]

In this study, we investigated for the first time whether BHVs employed for TAVI augment an α-Gal–specific humoral immune response of total immunoglobulin (Ig) G, IgG subclasses, and IgE; activate the complement system via C3a; induce citrullinated H3 (CitH3) as a marker for NET formation; and initiate the IL-33/ST2 pathway within 90 days after intervention compared with baseline levels. Patients receiving a MitraClip (Abbott Laboratories, Abbott Park, Ill) procedure served as controls (Figure 1 and Video 1).

METHODS

Ethics Approval

Ethics approval was obtained from the Institutional Ethics Committee of the Medical University of Vienna (EK 2218/2016) during June 2019. All experiments were performed in accordance with the approved ethical guidelines. Written informed consent was obtained from all study participants.

Study Design and Patients

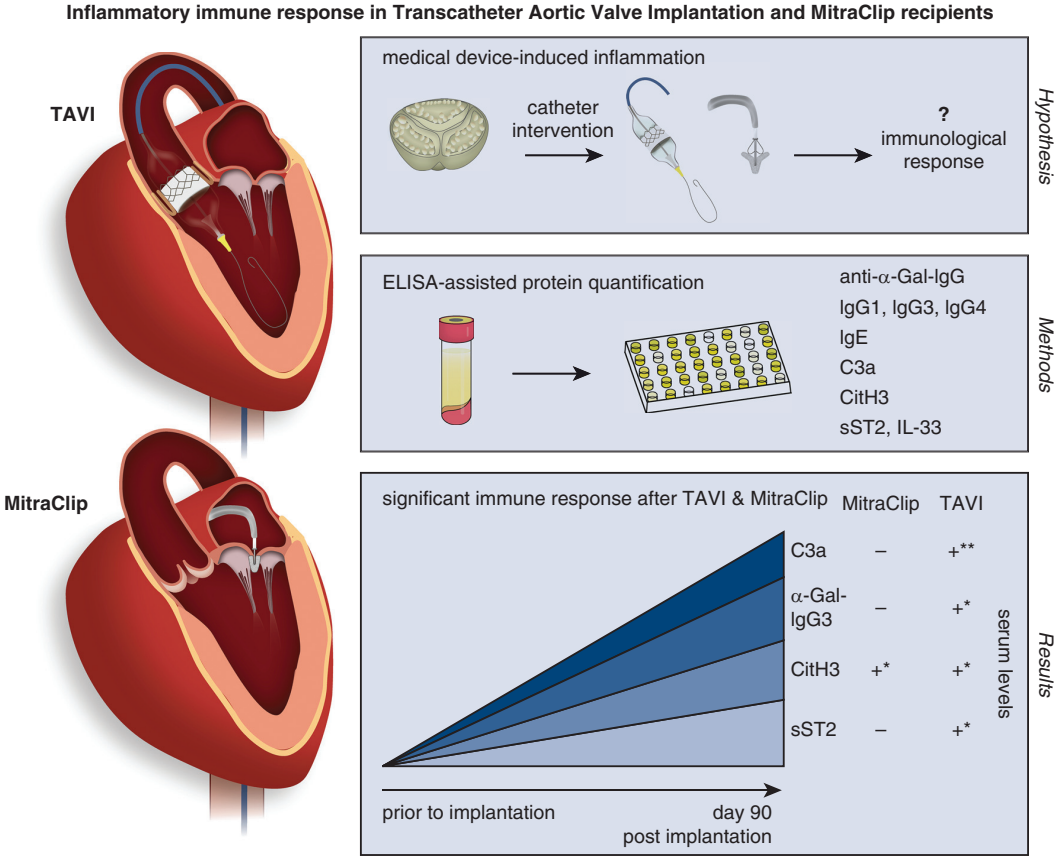
This work was designed as a prospective, observational, single-center study. Twenty-seven consecutive patients with severe aortic valve stenosis and 10 patients with severe mitral valve regurgitation undergoing TAVI or MitraClip procedures between March and August 2019 at the Department of Cardiology of the General Hospital Vienna (Medical University of Vienna) were prospectively analyzed. We excluded pregnant women, patients who were younger than age 18 years, and patients who did not give written informed consent. TAVI was performed in a hybrid operating room under general anesthesia or conscious sedation. Blood samples were drawn before the procedure and 90 days thereafter at a routine clinical check-up.

Enzyme-Linked Immunosorbent Assays

Microtiter plates (Maxisorp, Nunc, Denmark) were coated with Galα1.3-Galβ1–4GlcNAc-BSA (Dextra Laboratories, Reading, United Kingdom). Optimal coating concentrations of each protein were defined in preliminary experiments. Blocking was carried out using assay buffer (2.5 g human serum albumin, 500 mL phosphate buffered saline −/−, 250 μL Tween 20). Sera were diluted in phosphate buffered saline with 0.05% Tween-20 and 0.5% human serum albumin as follows: for IgE, 1:2; for IgG, 1:50; and for IgG1, 3, and 4, 1:20. After sample incubation and washing, the following horseradish peroxidase-conjugated detection antibodies were added: antihuman IgG-Fc (Sigma-Aldrich Corp, St Louis, Mo), IgG subclasses (Sigma-Aldrich Corp) and alkaline phosphatase-conjugated anti-human IgE (BD Bioscience Pharmingen, San Diego, Calif). A color reaction was obtained with peroxidase reagent tetramethylbenzidine (Sigma-Aldrich Corp) and the optical density was read at 450 nm using an absorbance microplate reader (Infinite F50; Tecan, Männedorf, Switzerland).[17]

A human C3a (BMS2089) enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen; Camarillo, Calif) was used for the quantitative detection of C3a following the manufacturer's instructions. Serum samples were diluted 1:50000 in assay buffer.

Cayman's CitH3 (Clone 11D3) ELISA kit (Cayman Chemical, Ann Arbor, Mich) was used to measure citH3 serum concentrations. The



α-Gal Gal = galactose-alpha-1,3-galactose, C3a = complement factor C3a, CitH3 = citrullinated histone H3, Ig = immunoglobulin, IL-33 = interleukin-33, sST2 = solube suppresion of tumorigenicity-2, *indicates $P < .05$, ** indicates $P < .01$

FIGURE 1. Inflammatory immune response in transcatheter aortic valve implantation (TAVI) and MitraClip (Abbott Laboratories, Abbott Park, Ill) recipients. Hypothesis: Biological medical devices for TAVI induce an galactose-alpha-1,3-galactose (α-Gal)–specific antibody dependent and antibody-independent immune response 3 months after implantation. Methods: Serum samples were drawn before and 90 days after TAVI and MitraClip and analyzed for α-Gal-specific immunoglobulin (Ig) G, IgG subclasses, IgE, complement factor 3a (C3a), citrullinated H3 (CitH3), soluble suppression of tumorigenicity-2 (sST2), and interleukin (IL)-33 using the enzyme-linked immunosorbent assay technique. Results: Significant immune responses were observed after TAVI in C3a, α-Gal-specific IgG3, citH3, and sST2 and MitraClip in citH3. – indicates no significance; + indicates significance.

microwell plate was coated with a monoclonal antibody (mAb) specific for histone H3 (citrullinated at R2, R8, and R17). Sera were added in a 1:2 dilution in assay buffer.

To assess sST2 and IL-33 serum concentrations, commercially available ELISA kits for sST2 (DY523B) and IL-33 (DY3625B) (R&D Systems, Minneapolis, Minn) were used. Sera were diluted 1:20 for sST2 and not diluted for IL-33.

Postinterventional Antithrombotic Therapy

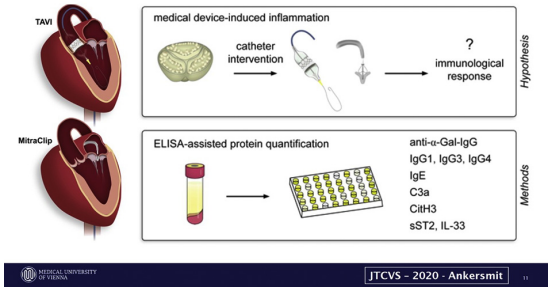
According to our institutional guidelines patients after TAVI/MitraClip procedure received dual antiplatelet therapy combining aspirin and a P2Y12 receptor inhibitor for 3 months, followed by lifelong daily low-dose aspirin (100 mg). In patients with preexisting anticoagulant therapy phenprocoumon or nonvitamin K antagonist oral anticoagulants were continued at a therapeutic dose.

Statistical Analysis

Before the study, we performed a power analysis using G*Power (Heinrich Heine University, Dusseldorf, Germany) according to a prior publication of our study group on α-Gal–specific IgG immune responses after surgical bioprosthetic valve implantation.[5]

To reveal a power of 99%, $\alpha = 0.05$, 2-sided, using the Wilcoxon-signed-rank test for matched pairs 27 patients undergoing TAVI were required. To calculate the size of the control group, the Wilcoxon-Mann-Whitney U test was used. To reach a power of 84%, $\alpha = 0.05$, 2-sided, we required 10 patients undergoing the MitraClip procedure.

Graphical methods (histograms) were employed to test normality. Data are reported as mean ± standard deviation for normally distributed data and median (25th percentile, 75th percentile) for nonnormal distributions. The Kruskal-Wallis rank test and Mann-Whitney U test were used for nonnormally distributed data and t tests were performed for



VIDEO 1. Inflammatory immune response in transcatheter aortic valve implantation (TAVI) recipients. Background: Clinical data on xenograft valve durability; galactose-alpha-1,3-galactose (α-Gal)–specific xenograft immune responses in cardiac surgery; α-Gal induced meat allergy and cardiac surgery. Study results: α-Gal xeongraft immune response in TAVI recipients, inflammation in TAVI recipients: soluble suppression of tumorigenicity-2 (sST2), NETosis, complement. Video available at: https://www.jtcvs.org/article/S2666-2736(21)00056-5/fulltext.

parametric data. The level of statistical significance was set at .05 (2-tailed *P* values). Statistical analyses were performed using SPSS software version 26 (IBM-SPSS Inc, Armonk, NY). GraphPad Prism 8 (GraphPad Software, La Jolla, Calif) was employed for data visualization (boxplots and line diagrams). Boxplots were designed as follows: box, first to third quartile; bar, median; whiskers, fifth to 95th percentile; all individual values are presented as dots.

Data Availability

All data generated or analyzed during this study are included in this article and Tables E1 through E4.

RESULTS

Demographic and Clinical Data

In our study, we enrolled 27 TAVI and 10 MitraClip patients, of whom 13 (48.1%) TAVI and 2 (20%) MitraClip patients were women. The median age of all patients was 78 years (range, 75-83 years) for TAVI and 76 years (range, 68-82 years) for MitraClip patients. Thirty-seven percent of all TAVI patients received bovine and 63% received porcine heart valves. The implanted medical devices are described in detail in Table E1. Left-ventricular ejection fraction increased and the concentration of N-terminal pro-brain natriuretic peptide (NT-proBNP) decreased statistically significantly in patients receiving TAVI 3 months after intervention ($P = .033$ and $P = .050$, respectively). Detailed baseline characteristics and clinical and echocardiography data are depicted in Tables 1 and 2. Inflammatory conditions in patients with hyperlipidemia and adult-onset diabetes mellitus are depicted in Tables E2 and E3. None of our patients experienced meat allergy before undergoing the TAVI/MitraClip procedure. Three patients had an allergy to penicillin, 1 patient was allergic to ciprofloxacin and 1 patient was allergic to band-aid.

TABLE 1. Demographic data

Characteristic	TAVI	MitraClip*	*P* value
Baseline characteristics			
Age (y)	78 (75, 83)	76 (68, 82)	.216
Female	13 (48.1)	2 (20.0)	.153
BMI	27.4 (23.9, 30.0)	24.5 (22.0, 31.1)	.428
Hypertension	20 (74.1)	8 (80.0)	1.000
Diabetes	15 (55.6)	3 (30.0)	.269
Hyperlipidemia	15 (55.6)	7 (70.0)	.481
Atrial fibrillation	9 (34.6)	5 (55.6)	.432
COPD	5 (18.5)	0 (0)	.295
CAD	19 (70.4)	8 (80)	.694
Smoker	5 (18.5)	2 (20.0)	.958
Bioprosthetic features			
Bovine tissue valve	10 (37)	–	–
Porcine tissue valve	17 (63)	–	–

Continuous, nonparametric values are presented as median (25th percentile, 75th percentile) based on Kruskal-Wallis test; categorical variables are presented as n (%) based on χ^2 test. *TAVI,* Transcatheter aortic valve implantation; *BMI*, body mass index; *COPD,* chronic obstructive pulmonary disease; *CAD*, coronary artery disease. *Abbott Laboratories, Abbott Park, Ill.

Augmented α-Gal–Specific IgG3 3 Months After TAVI

We investigated whether BHVs of a glutaraldehyde-fixed α-Gal–bearing scaffold installed via TAVI induce α-Gal–specific IgG and IgG subclasses (IgG1, IgG3, and IgG4) before and 3 months after catheter intervention.

We found significantly increased α-Gal–specific IgG3 serum concentrations in patients 3 months after TAVI compared with baseline levels ($P = .002$). Furthermore, we observed there is a trend toward augmented α-Gal–specific IgG, but not IgG1 or IgG4 (IgG, $P = .09$; IgG1, $P = .344$; and IgG4, $P = .279$). Neither α-Gal–specific IgG nor IgG subclasses (IgG1, IgG3, or IgG4) significantly increased in the MitraClip control cohort (Table 3).

Three months after TAVI, α-Gal–specific IgE serum concentrations did not increase statistically significant ($P = .284$). However, IgE sensitization occurred in 55% of all TAVI patients. Differences in α-Gal–specific antibodies between bovine and porcine heart valves for TAVI are shown in Table E4.

Significantly Increased C3a in TAVI Patients 3 Months After Intervention

The role of the complement system as innate immunity in xenograft rejection beyond naturally occurring cytotoxic α-Gal–specific mAbs is well described. Activation of the classical complement pathway is caused by the binding of antibodies to antigens and is the major mechanism of xenograft rejection.[8] We investigated whether BHVs for TAVI trigger systemic complement activation in vivo. We measured C3a because this protein is the hinge point of the alternative and lectin complement activation pathway.

Three months after TAVI, C3a levels were significantly increased compared with baseline levels (baseline, 7.8 μg/

TABLE 2. Clinical and echocardiographic data

Variable	TAVI			MitraClip*		
	Baseline	>3 mo	*P* value	Baseline	>3 mo	*P* value
Clinical						
NT-proBNP (pg/mL)	1716.0 (916.7, 4764.5)	1091 (760.5, 3388.0)	**.050**	2979.5 (1107.0, 7320.7)	1060.0 (635.0, 2984.0)	.314
Creatinine (mg/dL)	1.0 (0.93, 1.6)	1.0 (0.74, 1.3)	**.008**	1.1 (0.95, 1.8)	1.3 (1.0, 2.0)	.173
NYHA functional class ≥III	22 (81.5)	2 (7.2)	**.001**	7 (70)	1 (10)	**.002**
Echocardiographic parameters						
LVEF >55%	14 (51.9)	17 (63.0)		4 (40)	4 (40)	
LVEF 54%-45%	5 (18.5)	7 (25.9)	**.033**	1 (10)	3 (30)	.157
LVEF 44%-30%	4 (14.8)	3 (11.1)		4 (40)	2 (20)	
LVEF <30%	4 (14.8)	0 (0)		1 (10)	1 (10)	
sPAP (mm Hg)				64.0 (51.2, 78.2)	41.0 (30.0, 51.0)	.285
AV PPG	76.0 (65.5, 111.0)	16.5 (12.0, 26.5)	**.001**			
AV MPG	45.5 (41.7, 62.5)	9.5 (6.0, 15.0)	**.001**			
AV Vmax	4.6 (4.0, 5.5)	1.8 (1.6, 2.2)	**.018**			
AVA (cm^2)	0.7 (0.6, 0.85)	–	–			

Significant *P* values were written in boldface. Values are presented as median (25th percentile, 75th percentile) or n (%). *TAVI*, Transcatheter aortic valve replacement; *NT-proBNP*, N-terminal pro-B-type natriuretic peptide; *NYHA*, New York Heart Association; *LVEF*, left ventricular ejection fraction; *sPAP*, systolic pulmonary artery pressure; *AV*, aortic valve; *PPG*, peak pressure gradient; *MPG*, mean pressure gradient; *Vmax*, maximum velocity; *AVA*, aortic valve area. *Abbott Laboratories, Abbott Park, Ill.

mL; range, 3.2-37.9 μg/mL vs 3-month TAVI, 37.2 μg/mL; range, 4.6-89.3 μg/mL; $P = .001$). In contrast, patients receiving the MitraClip procedure had lower C3a serum concentrations after intervention compared with baseline values (baseline, 16.5 μg/mL; range, 5.7-48.5 μg/mL vs 3-month MitraClip, 6.3 μg/mL; range, 3.1-45.3 μg/mL; $P = .130$). These data are evidence that the implantation of glutaraldehyde-fixed biological scaffolds augments the activation of complement pathways in vivo (Figure 2, *A* and *B*). There were no differences in C3a serum concentrations between bovine and porcine heart valves for TAVI (Table E4).

Significantly Increased CitH3 Serum Concentrations, an Indicator of Granulocyte-Specific NETosis, 3 Months After TAVI and MitraClip Implantation

Neutrophils are an important cellular component of innate immunity. They play a critical role in microbial clearance, activation of other immune cells, and tissue damage and repair, and contribute to coagulation. Neutrophils are also involved in xenograft rejection. The presence of CitH3 in the serum is an accepted biological marker for the detection of granulocyte-specific NETosis.[18] Three months after TAVI, CitH3 was significantly higher than baseline levels (baseline, 2.7 ng/mL; range, 1.1-4.2 ng/mL vs 3-month TAVI, 3.9 ng/mL; range, 1.3-9.7 ng/mL; $P = .025$). Similar to the TAVI cohort, MitraClip patients also had significantly elevated CitH3 serum concentrations 3 months postintervention (baseline, 1.7 ng/mL; range, 1.5-5.2 ng/mL vs 3-month MitraClip, 2.9 ng/mL; range, 1.5-7.2 ng/mL; $P = .039$). These data indicate that TAVI, as well as MitraClip (to a lower extent) elicit NETosis in vivo (Figure 2, *C* and *D*). There were no differences in citH3 serum concentrations between bovine and porcine heart valves for TAVI (Table E4).

Significantly Increased sST2 but Not IL-33 Levels 3 Months After TAVI Implantation

Secretion of sST2 is triggered by the cytokines IL-1α, IL-1β, and IL-6 and contributes to the proinflammatory phase of systemic inflammation.[19] IL-33 itself can upregulate

TABLE 3. Serum concentrations of galactose-alpha-1,3-galactose (α-Gal)–specific antibodies in transcatheter aortic valve replacement (TAVI) and MitraClip (Abbott Laboratories, Abbott Park, Ill) recipients

Antibody	TAVI			MitraClip		
	Baseline	>3 mo	*P* value	Baseline	>3 mo	*P* value
IgG	11.5 (4.6, 14.9)	13.3 (6.4, 15.8)	.09	12.7 (7.2, 16.0)	8.0 (5.4, 12.4)	.193
IgG1	22.6 (34.7, 34.7)	29.7 (10.8, 48.5)	.344	59.6 (34.7, 70.)	58.03 (19.3, 74.5)	.556
IgG3	**5.3 (3.0, 4.6)**	**23.1 (6.1, 34.1)**	**.002**	21.8 (3.4-46.0)	6.3 (2.3, 20.7)	.232
IgG4	6.2 (2.3, 19.0)	6.1 (3.0, 58.0)	.279	6.2 (2.3-19.0)	6.1 (3.0, 58.6)	.910
IgE	0.51 (0.36, 1.1)	0.56 (0.42, 1.1)	.284	1.4 (0.32-7.7)	0.57 (0.27, 2.2)	.460

Significant *P* values were written in boldface. Optical density values are reported as median (25th percentile, 75th percentile). *Ig*, Immunoglobulin.

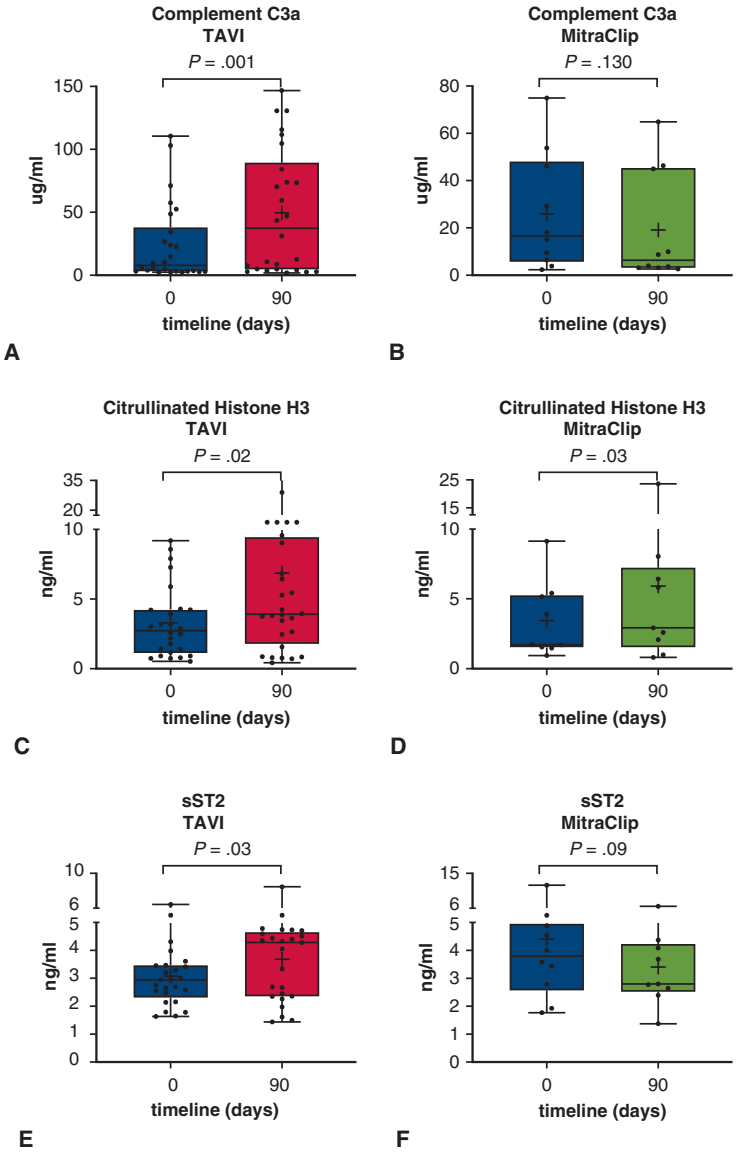


FIGURE 2. Significantly increased serum concentration of complement factor 3a (C3a), citrullinated H3 (CitH3), and soluble suppression of tumorigenicity-2 (sST2) 3 months after transcatheter aortic valve implantation (TAVI) compared with baseline levels. A, Three months after TAVI, C3a serum concentrations were significantly elevated compared to baseline levels. B, C3a serum concentrations did not increase 3 months after MitraClip (Abbott Laboratories, Abbott Park, Ill). C and D, Three months after TAVI and MitraClip, CitH3 serum concentrations were significantly upregulated compared with baseline levels. E and F, In TAVI patients, but not MitraClip patients, sST2 serum concentrations were significantly higher 3 months after intervention. For statistical analyses of serum concentrations between baseline levels and 3 months after TAVI or MitraClip the Wilcoxon matched-pairs signed-rank test was used.

expression of sST2. Aside from acting as a decoy receptor for IL-33, sST2 is believed to exhibit its anti-inflammatory capacity directly via inhibition of Toll-like receptor signalling, ultimately resulting in the downregulation of nuclear factor-kappa B in macrophages. Currently sST2 and IL-33 are emerging as markers of systemic inflammation with prognostic capacity in many clinical entities (eg, polytrauma, sepsis, and food

allergy).[14,15,20] Here, TAVI increased sST2 significantly (baseline, 2.9 ng/mL; range, 2.3-3.4 ng/mL vs 3-month TAVI, 4.3 ng/mL; range, 2.3-4.7 ng/mL; $P = .039$) compared with MitraClip implantation (baseline, 3.7 ng/mL; range, 2.5-4.9 ng/mL vs 3-month MitraClip, 3.2 ng/mL; range, 2.5-4.8 ng/mL; $P = .09$). IL-33 did not increase 3 months after TAVI or MitraClip compared with baseline levels (baseline, 25.58 pg/mL; range, 14.36-46.15 pg/mL vs 3-month TAVI, 25.01 pg/mL; range, 18.044-3.36 pg/mL; $P = .728$ and baseline, 55.39 pg/mL; range,11.79-806.0 pg/mL vs 3-month MitraClip, 17.92 pg/mL; range, 10.68-516.7 pg/mL; $P = .670$). These data show that α-Gal–bearing biological scaffolds induce a systemic host immune activation relevant to the compensatory anti-inflammatory response syndrome and allergic immune response (Figure 2, *E* and *F*). There were no differences in sST2 serum concentrations between bovine and porcine heart valves for TAVI (Table E4).

Clinical Outcome Data

Within 3 months after intervention 5 TAVI patients were readmitted to the hospital. Three patients were readmitted due to postintervention complications: One patient required vascular surgery of a pseudoaneurysm of the femoral artery after arterial puncture. Another patient developed a third-degree atrioventricular block and required a pacemaker implantation. Another patient was readmitted for gastrointestinal bleeding after receiving therapeutic anticoagulant therapy and aspirin. This patient received additional thromboembolic therapy due to atrial fibrillation and a coronary artery stent. The 2 noncardiology reasons for readmission were occurrence of a pseudoaneurysm in the left cubital artery after hemodialysis shunt graft implantation and dyspnea in a patient with a bronchial carcinoma. One MitraClip patient was readmitted for dyspnea. None of all included patients died within 3 months after TAVI/MitraClip intervention.

DISCUSSION

Here we demonstrate that TAVI elicits an up-regulation of α-Gal–specific IgG3 mAbs, activates the complement system, induces NET formation, and causes increased sST2/IL-33 cytokine spillage in vivo.

These data provide evidence that α-Gal–bearing medical devices are an ongoing inflammatory trigger. The link between α-Gal–specific antibodies and humoral valve destruction has seemed obvious since 2005, but until recently, there was mere conjecture by informed surgeons and allergologists.[4,5,21] Hawkins and colleagues[22] recently described 2 patients who underwent implantation of a BHV and developed a postoperative meat allergy associated with an α-Gal–specific IgE immune response. Both patients developed premature degeneration of the bioprosthesis that necessitated reoperation and implantation of a mechanical valve in the aortic position.[22] This was the first clinical proof that α-Gal on commercial BHVs and de novo development of α-Gal–specific IgE antibodies can lead to biovalve degeneration.

Platts Mills, FRS, was among the first allergologists to propagate the idea that formation of α-Gal–specific IgE is phenomenologically related to the development of meat allergy.[23] Kollmann and colleagues[17] extended this insight by showing that meat allergy is also associated with increased IgG, IgG1, and IgG3 directed against α-Gal. Relevant to the above findings is the notion that α-Gal–specific monoclonal antibodies remain rather stable in healthy humans.[24] In this study, we provide evidence that, similar to surgically implanted bioprostheses,[4,5] TAVI causes α-Gal–specific IgG3 production as well as de novo production of α-Gal–specific IgE. None of our older patients developed the symptoms described in meat-allergic patients.

The complement system and NETosis are known to interact reciprocally.[18] Opsonized antigens such as α-Gal are recognized by complement receptors on neutrophils, which subsequently induce NETosis, whereas neutrophils activate complement factors, especially the anaphylatoxins C3a and C5a, which can further alarm the immune system.[25]

Although our study found an α-Gal–induced enhancement of the complement system and NET formation within 3 months after TAVI, both systems were already known to be activated in stenotic aortic valves.[26] Helske and colleagues[27] found both elevated anaphylatoxin C3a and C5a levels and increased anaphylatoxin receptors, in particular C3a receptor, in stenotic aortic valves in contrast to nonstenotic valves.[27]

Implanted foreign bodies comprising biological scaffolds for TAVI and mechanical devices for MitraClip are both lifesaving interventions with an increased risk of thrombogenicity and bleeding by inducing flow alterations.[28] NETs activate the coagulation cascade directly and stimulate thrombosis in a platelet-dependent manner.[29,30] In our study, demonstrated enhanced citH3 serum concentrations in TAVI and MitraClip patients and thereby emphasize the importance of therapeutic antithrombotic therapy with either dual antiplatelet therapy with aspirin and a P2Y12 receptor inhibitors or anticoagulation in a therapeutic dose for patients after TAVI and MitraClip procedures.[31]

We also found that, in parallel to the humoral immune response, sST2 is significantly increased after TAVI implantation in the presence of significantly improved left ventricular function (according to New York Heart Association functional classification) and lower levels of NT-proBNP. sST2 is a biomarker of adverse outcomes after myocardial infarction and heart failure as well as systemic inflammatory conditions.[19,32,33] Our data, namely the increased sST2 levels 3 months after TAVI implantation with concomitantly reduced cardiac strain (as determined by lower levels of NT-proBNP), make it clear that sST2 may serve as a

marker of inflammation rather than heart failure in TAVI patients.

In 1987, Galili and colleagues[34] reported that the α-Gal epitope is present on cells of all mammals except for humans and Old World monkeys. However, valve hemodynamic deterioration was associated with porcine tissue valve implantation in patients undergoing surgical valve implantation. In our study, we did not find any differences in cytokine serum concentrations and anti-Gal antibodies between patients receiving bovine compared with porcine BHVs for TAVI. We therefore assume that porcine and bovine biological scaffolds display similar immunogenic potential. Further studies with higher sample sizes are warranted to evaluate species-specific immune responses elicited by xenogenic implants.

The link between α-Gal–specific inflammation and valve degeneration was determined through experimental work. Animal studies reported enhanced tissue calcification in rats and mice receiving α-Gal–positive xenogenic tissue implantation.[35,36] Our study group confirmed in humans short and midterm degradation of α-Gal–bearing cells of surgical BHVs through exposure to the human blood circuit. Explanted cells were double fluorescence labeled with IB4 against α-Gal residues and 4′,6-diamidino-2-phenylindole (DAPI) against DNA to stain for nucleated cells. A BHV explanted 1 week after implantation contained IB4/DAPI positive cells within the collagen matrix. In 2 patients, who underwent reoperation after 12 months, porcine tissue showed a complete lack of IB4/DAPI positive cells.[5]

Clinical studies confirmed surgical BHV degeneration 15 years postoperatively in 60% to 70% of all patients older than age 75 years, whereas 100% of all implanted BHVs fail within 5 years in patients younger than age 35 years.[37,38] Nevertheless, the 2017 Guidelines of the American Heart Association and the American College of Cardiology lowered the recommended age limit of BHV implantation to 50 years due to improved hemodynamic status, a lower risk of thromboembolic complications, and the absence of need for lifelong anticoagulant therapy compared with mechanical heart valves.[39] Besides, modern percutaneous valve-in-valve technologies provide less-invasive alternatives to treat potential BHV degeneration.[40]

We are convinced that TAVI will supersede surgical valve implantation in the future. Based on our data and data produced by others, currently utilized α-Gal–bearing biological scaffolds must be optimized by the commercial medical device industry. Several promising techniques have been reported to potentially increase the longevity of BHVs.[41,42] Already in 2013, treatment of BHVs with α-galactosidase was used to effectively remove α-Gal epitopes from both bovine and porcine tissues.[35] Naso and colleagues[43] introduced a preservation technique (ie, FACTA) that guarantees improved tissue biocompatibility by inactivating up to 95% of the α-Gal epitopes and thereby reducing the propensity of BHVs to calcify.

Besides preservation techniques, there is growing interest in developing Gal-free BHVs from Gal-knockout pigs. Recently, Rahmani and colleagues[44] used Gal-knockout pigs in engineering BHVs out of porcine pericardial leaflets with excellent hemodynamic parameters, long-term durability, and no thrombogenicity in a sheep model. Because BHVs for TAVI must be flexible, Gal-knockout pericardium xenografts seem to be favorable BHVs for TAVI to replace surgical aortic valve replacement in younger and lower-risk patients. Most recently, promising results of ongoing research concerning tissue-engineered heart valves for TAVI based on decellularized matrix in the pulmonary and aortic tissue were published.[45]

Our study has several limitations due to the limited sample size. We compared 2 different pathologies and surgical interventions: patients with aortic stenosis undergoing TAVI and patients with severe mitral regurgitation undergoing the MitraClip procedure. Due to the small sample size, we might have missed important demographic and immunological differences between groups. Further, we did not include patients after surgical aortic valve replacement as a control group. We could only draw our conclusions on similarities in the inflammatory response after surgical aortic valve replacement and TAVI patients due to prior research of our study group.[4,5] Further, according to the current guidelines and institutional standards of the TAVI procedure, the median age of our study cohort was 78 years. We therefore cannot draw conclusions on systemic inflammatory responses in younger patients. In addition, determining immunological changes after prolonged follow-up periods might help to reveal whether these inflammatory changes will persist and have any effects on clinical outcomes and valve durability.

CONCLUSIONS

TAVI significantly improved left-ventricular function and reduced clinical symptoms in patients within 3 months after intervention. We present evidence that TAVI implantation elicits an α-Gal-specific and unspecific humoral systemic inflammation that may influence BHV durability. We believe that the medical community should be cautioned against the uncritical lowering of age limits in recipients of α-Gal–bearing TAVI devices.[46]

Conflict of Interest Statement

The authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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TABLE E1. Implanted medical devices for transcatheter aortic valve implantation and MitraClip (Abbott Laboratories, Abbott Park, Ill)

Device	n	Bovine/porcine
Transcatheter heart valves		
Edwards, Sapien 3 Ulta transcatheter aortic valve	10	Bovine
Medtronic, CoreValve Evolut PRO transcatheter aortic valve	4	Porcine
Boston Scientific, ACURATE neo Aortic Valve	8	Porcine
Abbott, Portico valve	1	Porcine
St Jude Medical, Portico valve	4	Porcine
MitraClip devices		
Edwards, PASCAL transcatheter valve repair system	2	
Abbott, MitraClip XTR Clip Delivery System	5	
Abbott, MitraClip NTR Clip Delivery System	3	

TABLE E2. Baseline inflammatory conditions in patients with hyperlipidemia and adult-onset diabetes mellitus

Hyperlipidemia	Yes	No	*P* value
IgG (OD)	11.6 (3.2, 14.9)	11.7 (5.1, 15.8)	.710
IgG1 (OD)	27.3 (8.5, 70.6)	38.2 (15.1, 70.8)	.477
IgG3 (OD)	2.3 (2.0, 6.8)	2.0 (1.7, 4.8)	.115
IgE (OD)	0.5 (0.4, 2.0)	0.9 (0.3, 1.6)	.988
sST2 (ng/mL)	2.8 (2.2, 3.5)	3.4 (2.6, 4.3)	.414
c3a (μg/mL)	12.1 (3.1, 30.5)	10.5 (3.8, 53.7)	.496
citH3 (ng/m)	2.8 (1.4, 6.2)	2.8 (1.1, 4.2)	.567
Adult-onset diabetes mellitus			
IgG (OD)	12.6 (6.6, 14.8)	11.7 (4.6, 15.6)	.832
IgG1 (OD)	33.7 (19.3, 70.6)	38.2 (2.9, 70.7)	.564
IgG3 (OD)	2.1 (1.8, 3.9)	2.4 (1.8, 6.4)	.496
IgE (OD)	0.6 (0.4, 1.7)	0.5 (0.3, 1.8)	.564
sST2 (ng/mL)	3.2 (2.6, 4.4)	2.7 (0.0, 3.5)	.330
c3a (μg/mL)	12.1, (4.3, 53.7)	10.5 (3.2, 34.3)	.523
citH3 (ng/mL)	2.8 (1.3, 6.7)	2.8 (1.4, 5.1)	.542

Values are reported as median (25th percentile, 75th percentile). *Ig*, Immunoglobulin; *OD*, optical density; *sST2*, soluble suppression of tumorigenicity-2; *c3a*, complement factor 3a; *citH3*, citrullinated H3.

TABLE E3. Fold increase of serum cytokine levels and galactose-alpha-1,3-galactose–specific antibodies in patients with hyperlipidemia and adult-onset diabetes mellitus

Hyperlipidemia	Yes	No	*P* value
IgG	1.7 (−19.5, 56.9)	−3.1 (−22.5, 19.9)	.386
IgG1	−8.1 (−52.1,16.7)	−1.5 (−48.6, 30.5)	1.000
IgG3	40.0 (−13.2, 92.5)	13.5 (−3.5, 47.7)	.496
IgE	16.0 (−34.1, 63.7)	−2.0 (−17.6, 72.7)	.781
sST2	25.3 (−20.5, 74.2)	−1.9 (−28.5, 38.6)	.224
c3a	29.3 (−66.0, 62.0)	3.4 (−16.0, 48.5)	.926
citH3	20.6 (−17.9, 61.8)	11.4 (−17.0, 68.1)	.710
Adult-onset diabetes mellitus			
IgG	5.1 (−20.6, 52.4)	−3.5 (−22.2, 20.8)	.564
IgG1	0.0 (−36.9, 10.9)	−11.4 (−49.9, 47.0)	.801
IgG3	51.0 (−3.9, 155.0)	13.5 (−17.9, 47.7)	.145
IgE	30.3 (−17.1, 30.3)	−2.0 (−61.3, 39.2)	.083
sST2	26.1 (−24.8, 48.9)	−3.5 (−22.2, 20.8)	.704
c3a	20.4 (−57.6, 52.1)	3.4 (−21.0, 63.4)	1.000
citH3	33.9 (−12.8, 72.1)	11.4 (−19.7, 40.0)	.391

Values are reported as median (25th percentile, 75th percentile). *Ig*, Immunoglobulin; *OD*, optical density; *sST2*, soluble suppression of tumorigenicity-2; *c3a*, complement factor 3a; *citH3*, citrullinated H3.

TABLE E4. Differences in cytokine serum concentrations and galactose-alpha-1,3-galactose–specific antibodies between bovine and porcine heart valves for TAVI

Variable	Bovine	Porcine	*P* value
IgG	8.3 (−3.8 to 141.8)	1.4 (−20.6 to 53.9)	.315
IgG1	−5.6 (−40.6 to 46.1)	3.1 (−98.1 to 65.1)	.953
IgG3	43.5 (−11.2 to 101.8)	37.5 (−1.6 to 156.7)	.841
IgE	−3.5 (−58.6 to 84.3)	3.1 (−98.1 to 65.1)	.514
sST2	19.7 (−12.8 to 32.7)	21.2 (−17.2 to 35.2)	.777
c3a	−9.5 (−12.8 to 52.7)	45.5 (10.0 to 67.3)	.056
citH3	−0.4 (−65.7 to −33.7)	11.1 (−14.1 to 74.0)	.176

Values are reported as median (interquartile range). *Ig*, Immunoglobulin; *sST2*, soluble suppression of tumorigenicity-2; *c3a*, complement factor 3a; *citH3*, citrullinated H3.

CHAPTER EIGHT: DISCUSSION

7.1 General Discussion

The alpha-Gal epitope was mentioned for the first time by Galili and co-workers in 1987.[451] A few years thereafter, an association between alpha-Gal–specific antibodies and hemodynamic deteriorations of newly implanted bovine or porcine BHVs became evident. [30, 31, 574] While the impact of surgical BHVs on the immune system was studied over many years, the role of alpha-Gal–bearing medical devices for TAVR on the immune system remained undiscovered until recently. In this study we therefore focused on immune activation after TAVR and found that the TAVR procedure induces alpha-Gal–specific IgG3 antibodies, enhances the complement system, activates NET formation, and up-regulates sST2/IL-33 cytokine expression in patients' blood. These findings were evident after implantation of both porcine and bovine BHVs, manufactured for the TAVR procedure.

Surgical BHV degradation of alpha-Gal–bearing heart valves was already discovered in 2005 by our study group through the following experiment: Nucleated cells of explanted BHVs were stained via double fluorescence labeling with IB4 against alpha-Gal and 40, 6-diamidino-2-phenylindole (DAPI). One BHV was already explanted within a week after implantation. In this heart valve IB4/DAPI positive cells could be detected within the collagen matrix. In contrast, in another BHV explanted one year after surgical BHV implantation, IB4/DAPI positive cells were completely lacking.[31] These findings provided the basis for many other considerations. Today, it is already well accepted that surgical BHVs degenerate in an age dependent-manner. Especially in younger patients, aged below 35 years, BHVs are known to degenerate more rapidly.[575, 576] Since, modern BHVs provide a better hemodynamic picture compared to mechanical heart valves with a reduced risk of thromboembolic complications, no lifelong anticoagulant therapy is longer required. As a consequence, the guidelines of the AHA/ACC of 2017 lowered the recommended age limit for surgical BHV implantation to 50 years.[555] Furthermore, valve in- valve TAVR provides a new treatment option for degenerated surgical BHVs.[577] Thus, Traxler and colleagues recently analyzed long-term outcome of mechanical aortic valve compared to BHV recipients in Austria between 2010 and 2018. In total 13 993 patients were studied. The study group found better long- term survival, a lower risk of reoperation and myocardial infarction and no increased stroke rate in younger patients, aged between 50– 65 years, who received mechanic aortic valve prostheses. [578] The classical pathway of the complement system and NETosis work closely togehter.[579] On the one hand,

neutrophils stimulate complement factors, most importantly C3a and C5a, which further enhance an immune response. On the other hand, complement receptors on the surface of neutrophils recognize opsonized antigens such as the alpha-Gal epitope and subsequently induce NET formation.[580] NET formation is characterized by neutrophil chromatin, which is decorated with antimicrobial proteins to catch and kill pathogens.[579] In our study, solely patients diagnosed with severe AS were treated with TAVR. At this point it is important to mention that the complement and the NET formation system are known as enhanced in AS patients.[581, 582] We could reproduce these findings in contrast to patients receiving the TEER procedure. In our study patients with severe AS, but not patients with severe MR citH3 serum levels were already significantly augmented at baseline, prior to intervention.

As reported by Kollmann et al. and Platts Mills et al. alpha-gal specific IgE, IgG, IgG1 and IgG3 are known to be present in sera of subjects with meat allergy.[483] [489] More recently a research group found a link between meat allergy and the an immune response to alpha-Gal carrying BHVs. The study group reported of two cases that developed a meat allergy with an alpha-Gal-specific IgE immune response after BHV implantation. Both BHV recipients developed premature degeneration of the BHV and required a redo surgery.[583] However, the number of patients developing a meat allergy after valve implantation seems very little considering that there are more than 280 000 implantations each year; half of them BHVs.[584] In our study none of our patients developed a meat allergy after TAVR. In line with these findings, there was no statistically significant increase of alpha-Gal specific IgE antibodies.

According to literature, NETs exert pro-thrombotic effects by stimulating the coagulation system and activating platlets.[585, 586] In our research work, citH3 serum levels were elevated in both, TAVR and TEER patients. We therefore concluded that both procedures carry an elevated risk of thrombogenicity, but also bleeding due to flow alterations.[587] Therefore, therapeutic antithrombotic therapy is an important part of successful TAVR and TEER.[588] sST2 has emerged recently as a promising biomarker of myocardial stress.[89] Studies reported that sST2 serum concentrations are increased in patients with myocardial infarction, congestive heart failure and AS.[589, 590]

In our research work, sST2 increased significantly three months after TAVR. Additionally, left ventricular function improved clinically over the same time period, as determined via the New York Heart Association functional classification and NT-proBNP levels. We therefore concluded that sST2 may rather serve as an inflammatory marker than pointing out heart failure. There is on-going research on improving these biological

scaffolds, due to limited life durability of currently utilized BHVs determined by our and several other study groups.[490, 591] Especially in an attempt to decrease the anti-BHV immune response in young recipients, decellularization was introduced as a part of processing of the porcine BHV.[592] However, decellularization did not avoid AB production against the porcine valve antigens. In addition, decellularization caused substantial loss in valve stiffness, and resulted in significant ECM disruption.[490, 494, 593] Another attempt to improve valve durability, suggested to delay BHV impairment by eliminating immunogenic carbohydrate antigens from the BHV, such as alpha-gal and Neu5Gc.[501, 594, 595] An Italian study group invented FACTA, a preservation technique to inactivate up to 95% of the alpha-Gal epitopes to improve biocompatibility and to reduce BHV calcification and degeneration.[596] Furthermore, Gal-free BHVs, produced out of porcine pericardial leaflets of alpha-Gal knockout pigs, were studied in a sheep model.[597] While, removal of the carbohydrate antigen eliminated the immune response against alpha-Gal, several other immunogenic porcine antigens remained unaffected.[490]

While TAVR remain dependent on a fully collapsible BHV, alternative surgical aortic valve repair/replacement can be performed wit complete avoidance of BHVs comprising the Ross or the Ozaki procedure.[598, 599] The Ozaki procedure is known as aortic valve neo-cuspidization with fixed autologous pericardium as an effective treatment of aortic valvulopathies with better hemodynamics compared to biological aortic valve replacement and without the need for lifelong anticoagulation.[600] The Ross procedure is also known as the pulmonary autograft procedure involves the replacement of the non-functioning aortic valve by the pulmonary valve and the original pulmonary valve by a healthy donor valve.[599]

7.2 Limitations of this study

This present cohort study includes several drawbacks owing to the small sample size. As a result, we might have missed decisive clinical and immunological distinctions between the TAVR and the TEER group. Furthermore we compared not only two different pathologies, but also two different interventions. Furthermore we did not compare TAVR to SAVR patients; since we could draw our conclusions based on our historic SAVR group of our previously published data.[30, 31] Besides, the median of our age group was 78 years old. Therefore, our measured systemic inflammatory response cannot automatically be adopted for younger patients. Additionally, long-term data on alpha-Gal specific and unspecific humoral immune responses to TAVR remain outstanding.

7.3 Conclusion

In summary, TAVR ameliorates left ventricular function and alleviates clinical symptoms within 3 months after the procedure. In this study, we brought further evidence that TAVR elicits an alpha-Gal specific and unspecific humoral systemic inflammatory response that may impact on BHV durability. Further research on BHV durability after TAVR in younger patients remains warranted.[601]

CHAPTER NINE: METHODS

The following information has been essentially reproduced verbatim from my publication “Inflammatory immune response in recipients of transcatheter aortic valves” in 2021.

8.1 Ethics Approval

Ethics approval was obtained from the Institutional Ethics Committee of the Medical University of Vienna (EK 2218/2016) in June 2019. All experiments and analyses were performed in accordance with the approved ethical guidelines. Written informed consent was obtained from all participating patients.

8.2 Study Design and Patients

This study was designed as a prospective, observational, single-center cohort study. Twenty-seven consecutive patients diagnosed with severe AS and 10 patients with severe MR planned for TAVR or TEER intervention were prospectively investigated. All investigated patients received their procedure between March and August 2019 at the Department of Cardiology of the General Hospital Vienna (Medical University of Vienna). Pregnant women, patients who were younger than 18 years, and patients who did not give written informed consent were excluded of this study. Both procedures were performed in a hybrid operating room under general anaesthesia or conscious sedation. Blood samples were drawn prior to the procedure and 90 days thereafter at a routine clinical check-up.

9.3 Enzyme-Linked Immunosorbent Assays

We coated microtiter plates (Maxisorp, Nunc, Denmark) with Gala1.3-Galb1–4GlcNAc-BSA (Dextra Laboratories, Reading, United Kingdom). Optimal coating concentrations were already defined in preliminary experiments by our study group. Blocking was performed with assay buffer (2.5 g human serum albumin, 500 mL phosphate buffered saline _/_, 250 mL Tween 20). Sera were diluted in phosphate buffered saline with 0.05% Tween-20 and 0.5% human serum albumin: for IgE, 1:2; for IgG, 1:50; and for IgG1, 3, and 4, 1:20. After sample incubation and washing, horseradish peroxidase-conjugated detection antibodies were added such as antihuman IgG-Fc (Sigma-Aldrich Corp, St Louis, Mo), IgG subclasses (Sigma-Aldrich Corp) and alkaline phosphatase-conjugated anti-human IgE (BD Bioscience Pharmingen, San Diego, Calif). A color reaction was

obtained adding peroxidase reagent tetramethylbenzidine (Sigma-Aldrich Corp). The optical density was read at 450 nm using an absorbance microplate reader (Infinite F50; Tecan, M€annedorf, Switzerland).[484] We measured C3A serum concentrations using a human C3a (BMS2089) enzyme-linked immunosorbent assay (ELISA) kit (Invitrogen; Camarillo, Calif) following the manufacturer's instructions. We diluted serum samples 1:50000 in assay buffer. To quantify CitH3 serum concentrations Cayman's CitH3 (Clone 11D3) ELISA kit (Cayman Chemical, Ann Arbor, Mich) was used. The microwell plate was already coated with a mAB specific for histone H3 (citrullinated at R2, R8, and R17). Sera were added, diluted 1:2 dilution in assay buffer. sST2 and IL-33 serum concentrations were assed using commercially available ELISA kits for sST2 (DY523B) and IL-33 (DY3625B) (R&D Systems, Minneapolis, Minn). Sera were added in 1:20 dilution for sST2 and without dilution for IL-33.

8.4 Postinterventional anticoagulation and antiplatelet therapy

According to our institutional standards patients received dual antiplatelet therapy combining aspirin and a P2Y12 receptor inhibitor for a minimum of three months after TAVR or TEER procedure, followed by lifelong daily low- dose aspirin (100 mg). In patients with pre-existing anticoagulation with phenprocoumon or nonvitamin K antagonist, oral anticoagulants were continued at a therapeutic dose.

8.5 Statistical Analysis

Before initiation of this study, we performed a power analysis using G*Power (Heinrich Heine University, Dusseldorf, Germany) according to a previous, published investigation on alpha-Gal–specific IgG immune reactions post surgical bioprosthetic valve implantation.[31] To reveal a power of 99%, a α=0.05, 2-sided, using the Wilcoxon- signed-rank test for matched pairs 27 patients undergoing TAVR were necessary. To calculate the size of the control group, the Wilcoxon-Mann- Whitney U test was applied. To reach a power of 84%, a α=0.05, 2-sided, we had to include 10 patients undergoing the TEER procedure. Graphical methods such as histograms were plotted to test normality. Data were reported as mean ± standard deviation for parametric data and median (25th percentile, 75th percentile) for non-parametric distributions. The Kruskal-Wallis rank test and Mann-Whitney U test were applied for non-parametric and t-tests were performed for parametric data. The level of statistical significance was set at 0.05 (2- tailed P values). Statistical analyses were performed using SPSS software version 26 (IBM-SPSS Inc,

Armonk, NY). GraphPad Prism 8 (GraphPad Software, La Jolla, Calif) was employed to visualize our findings (boxplots and line diagrams). Boxplots were designed with a box, first to third quartile; bar, median; whiskers, fifth to 95th percentile; individual values were presented as dots.

Epilogue

Transiently introduced synthetic non-degradable materials during major thoracic surgery comprising ECMO and CPB insertion during LUTX and PEA initiate a temporary immune response limited on the intraoperative period, confounded by major surgery. When performing LUTX, it remains therefore important to think about the two key questions. 1) Should synthetic non-degradable mechanical assist devices be routinely applied during LUTX or should these devices be reserved for selected, unstable patients and 2) if mechanical assist devices are applied should ECMO be preferred over CPB. Even though, taking the inflammatory trigger of these membranes into account, there is consent in the scientific community of this field that ECMO support provides more advantages than disadvantages during thoracic surgery by enabling completely stable intraoperative circumstances, avoiding the “first lung syndrome” and controlling reperfusion for initial graft function.[602] At our institution in a retrospective analysis showed improved 1-,3- and 5-year survival in patients undergoing LUTX on ECMO compared to off-pump surgery. This phenomenon even remained after propensity score-matched analysis.[603] Further, multiple studies comparing ECMO and CPB during LUTX reported superiority of ECMO over CPB.[154, 155, 604] The use of CPB was associated with an increased transfusion rate of packed red blood cells, a higher rate of patients requiring hemodialysis, bleeding revisions and prolonged mechanical ventilation, ICU and hospital stay.[604, 605]

Permanently introduced biological non-degradable materials, like BHVs for TAVR, trigger on-going inflammation for at least three months. In line with ECMO support during LUTX the duration of exposition to biomaterials seems decisive for the period of enhanced inflammation. While, reducing time of surgery and time of ECC can shorten the time-span of ECMO support, BHVs seem to be a life-long inflammatory trigger. Therefore, only further improvements of the commercially BHVs as already proposed by Galili in his new review on in situ ”humanization” or the complete avoidance of BHVs by performing liberally alternative surgeries comprising homograft implantations, the Ross or the Ozaki procedure can solve the problem of life-long inflammation. [598, 599]

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Curriculum Vitae

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Specialist examination

2021	European Diploma in Anaesthesiology and Intensive Care examination (**EDAIC I**)
2021	Österr. Facharztprüfung für Anästhesie

Postgraduate Education

2022-	Division of Cardiac Thoracic Vascular Anaesthesia and Intensive Care Medicine, Medical University of Vienna
2018-2022	Division of General Anaesthesia and Intensive Care Medicine, Medical University of Vienna
2017- 2018	Division of Cardiac Thoracic Vascular Anaesthesia and Intensive Care Medicine, Medical University of Vienna
2017	Cardiac surgery, Medical University of Vienna
2017	Division of Cardiac Thoracic Vascular Anaesthesia and Intensive Care Medicine, Medical University of Vienna

Education

2016	Medical school graduation, Medical University of Innsbruck

Teaching activity

2022	Changes in neutrophil markers and cytokines during thoracic surgeries: An investigation of the concomitant inflammatory response. "Diploma thesis supervision"

2020/2019/2018	Themenspezifische Untersuchungstechniken
2020/2019/2018	Ernährung und Verdauung
2020/2019/2018	Notfall- u. Intensivmedizin
2018	Physikalische Gesundenuntersuchung

Presentations

2022	Increased suPAR serum concentrations in patients with left ventricular remodeling - a consequence of aortic valve stenosis. EACTAIC, Neapel, Italy
2022	Resting energy expenditure impacts on short- and long-term mortality in critically ill patients after cardiac surgery- a retrospective analysis. EACTAIC, Neapel, Italy
2021	Den RQ bewerten: IZI Kongress Hernstein. Vienna, Austria
2020	Ab in die Praxis: Indirekte Kalorimetrie. Vienna, Austria
2020	The lost immunological innocence of biological scaffolds for TAVI. EACTS ,Barcelona, Spain.
2020	Lipocalin-2 - A novel biomarker for Restrictive Allograft Syndrome. EACTS ,Barcelona, Spain.
2019	Inflammatory consequences after ECMO: preliminary data. EACTS. Lisbon, Portugal.
2019	Nutritionday ICU: Annular Change in Onset of Parenteral Nutrition. ESPEN. Warsaw, Poland.
2019	Fallbericht: Kalorimetrie nach Herz-OP. Nutrition Update **2019** in Krems, Austria.
2019	Clinical evaluation of the ease of use of a new indirect calorimeter (Q-NRG) for energy expenditure measurement in ICU patients. Postgraduales Curriculum HTG. Vienna, Austria.
2018	The impact of non-pulsatile perfusion on cerebral blood flow and brain oxygenation. EACTA.Manchester. England

Original manuscripts

Cecilia Veraar, Arabella Fischer, Martin H Bernardi, Isabella Sulz, Mohamed Mouhieddine, Martin Dworschak, Edda Tschernko, Andrea Lassnigg, Michael Hiesmayr. Absent Metabolic Transition from the Early to the Late Period in Non-Survivors Post Cardiac Surgery. Nutrients. **2022** Aug 17;14(16):3366. doi: 10.3390/nu14163366.

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Abstracts

Follistatin-like 1 and biomarkers of neutrophil activation are associated with ooor outcome after lung transplantation on VA-ECMO. AIC **2022** in Bregenz, Austria

Increased suPAR serum concentrations in patients with left ventricular remodeling - a consequence of aortic valve stenosis. EACTAIC **2022** in Vilnius, Lithuania

Resting energy expenditure impacts on short- and long-term mortality in critically ill patients after cardiac surgery- a retrospective analysis. EACTAIC **2022** in Vilnius, Lithuania

Age modifies clinical nutrition choices independently of food intake in the wards: a nutritionday 2006-15 analysis. ESPEN **2022** in Vienna, Austria

How does a previous ICU stay modify nutrition support in the wards: a risk adjusted evaluation from 191 886 adult patients from Nutritionday 2006-2019. ESPEN **2021,** virtual congress.

Uncoupling of cerebral blood flow and brain oxygen saturation under non-pulsatile flow conditions. EACTA **2019** in Ghent, Belgium

Awards

EACTA 2018 in Manchester- 3rd Prize –The impact of non-pulsatile perfusion on cerebral blood flow and brain oxygenation (best oral presentation).