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Direktor: Prof. Dr. med. L.-H. Boldt

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Hemodynamic mechanisms of experimental and clinical heart failure: a translational perspective

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Dr. Alessio Alogna

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Dekan: Prof. Dr. Axel R. Pries

1. Gutachter: Prof. Dr. med. Christian Schulze

2. Gutachter: Prof. Dr. med. Andreas Schäfer

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1. Introduction

1.1 Heart failure: clinical and hemodynamic definition

Heart failure (HF) is a highly-prevalent (1,2) clinical syndrome consisting of reproducible symptoms (shortness of breath and fatigue overall) and accompanied by objective signs (e.g. peripheral oedema and pulmonary crackles) (3). HF is defined as the inability of the heart to meet body oxygen demand at rest or exercise, or to require an elevation in cardiac filling pressures in order to do this (4). Elevated left ventricular (LV) filling pressures play therefore a central role in this definition, and can be readily assessed in the cardiac catheterization laboratory. However, cardiac catheterization is invasive and time-consuming, so that the physicians, beside clinical examination, rather rely in the daily clinical routine on non-invasive assessment of cardiac function based on 12-lead electrocardiogram, blood biomarkers, echocardiography, chest X-ray and, in a selected subgroup of patients, implantable devices, such as pulmonary pressure sensors (5).

Most importantly, according to the most recent ESC HF guidelines (3) and the recent universal definition of HF (6), HF patients are classified according to their LV ejection fraction (LV EF) in 3 different phenotypes: patients with reduced (≤ 40%, HFrEF), mildly reduced (41 to 49%, HFmrEF) and preserved (≥50%) LV EF (HFpEF) (3). HF is usually divided into two presentations: chronic heart failure (CHF) and acute heart failure (AHF)(3), depending on the diagnosis (already established in chronic vs not yet in acute patients) and on the onset of symptoms (gradual onset in chronic vs rapid onset in acute patients). The symptoms are conventionally described according to the New York Heart Association (NYHA) functional classification, divided into class I up to class IV with increasing severity of symptoms and their association with physical activity. However, this classification predicts prognosis of HF patients worse than other indicators, while patients with mild symptoms may still own a high risk of hospitalization and mortality (7). Once the HF diagnosis has been established, further investigations underlying the aetiology are needed, in order to detect potentially reversible and/or treatable causes of HF. The recommendations for advance diagnostic tests include imaging modalities such as exercise or pharmacological stress echocardiography, Coronary angiography with or without right heart catheterization, cardiac magnetic resonance (CMR) imaging, computed tomography coronary angiography (CTCA) and single-photon emission CT (SPECT) (3).

Despite important therapeutic advances in the last year, especially in the field of HFrEF, with several landmark clinical trials impacting on hard endpoints such as hospitalization and

cardiovascular mortality (8-11), HF is still characterized by poor quality of life and high mortality (3). This is certainly related to manifold causes, and the ESC guidelines 2021(3) nicely summarizes, under paragraph 16, the so called “gaps in evidence”, which are unknowns, which potentially contribute to impaired outcomes. A central thematic core of the current translational work refers to following gaps: i) understanding of underlying characteristics, pathophysiology, and diagnosis of HF and ii) development of specific therapies according to phenotypes. In particular, the studies related to aims 1 to 3 and 5 of this work are describing non-invasive and invasive hemodynamic indices of LV function both in preclinical and clinical settings, while aim 4 is dedicated to the establishment of a novel, inhalative therapy for chronic HFrEF based on a nanocarrier platform.

1.2 Heart failure and cardiac work: the heart as a hydraulic pump.

The classification of HF patients based on their LV EF is widely accepted and this concept is reflected by the most recent guidelines (3). Firstly described by a later-in-life eminent psychiatrist from New York City, Stuart Bartle, at the American College of Cardiology meeting in 1965 (12), LV EF has being shaping inclusion criteria and study design of decades of clinical trials in HF. However, this classification has obviously limitations, including the lack of a clear correlation with HF pathognomonic pathophysiological features (12,13). Indeed, the EF is a parameter which reflects rather LV geometry than LV systolic function, as already debated early after its appearance in literature (14). Most of the echocardiographic parameters currently used in the clinical routine to characterize LV systolic function, such as LV EF and LV global longitudinal strain (GLS), do not take into account the pressure developed within the LV, and therefore fall too short in describing LV function as a hydraulic pump (15).

The gold standard for the assessment of LV function is pressure-volume (PV) analysis (16), allowing to describe systolic and diastolic proprieties in a reasonably load-independent fashion (15) via end-systolic and end-diastolic PV relationships. Furthermore, plenty of information on myocardial energetics and efficiency can be derived by PV analysis, such as the so called PV area (PVA), which is defined as the sum of stroke work (SW) and the potential energy (PE) (17,18). SW represent the external work of the heart, i.e. the energy required for blood ejection in the peripheral vasculature. PE is the energy the myocardium needs to develop and store in the myofilaments, in order to allow the SW to happen, while the ratio between SW and PE represents the efficiency of this process. However, due to its invasive nature and complexity, the concept of external work of the heart has struggled being implemented into daily clinical practice, especially with regard to CHF patients.

In the past years, novelties in this field of research have opened new scenarios for the management of HF patients. First of all, in the field of AHF, several investigators have reported on the role of the so called cardiac power output (CPO), a parameter conceptually overlapping with the abovementioned PV-derived myocardial energetics indices, i.e. pressure volume area. CPO is assessed as the product of cardiac output (CO) and mean aortic pressure (MAP), divided by a constant of 451 and was shown to be a strong predictor of cardiovascular mortality in cardiogenic shock patients (19). The physical unit of CPO is expressed in Watt (W), therefore expressing the energy consumption of the heart per unit of time. Interestingly, peak exercise CPO appears to

strongly correlate with outcomes in CHF patients (15). Secondly, the development of non-invasive pressure-strain loops, based on the combination of LV strain analysis, as a surrogate for LV volumes, and simple non-invasive brachial pressures for the estimation of LV pressure signals, allows now to approximate PV analysis as good as ever before (15).

Aim 1 of this work focuses on the relationship between LV CPO, conductance-derived LV SW and several classical parameters of LV function under various inotropic states in experimental heart failure. Aim 2 of this work focuses on the reproducibility of a novel and quick cardiac MR feature tracking analysis (CMR-FT) of LV strain based on conventional cine balanced steady state free precession (b-SSFP) images in pigs. Aim 3 compares invasively measured LV CPO and end-systolic elastance (Ees, slope of the end-systolic pressure-volume relationship) with the abovementioned CMR-FT derived LV global strain indices, with and without taking into account LV pressure in pigs.

1.3 Hear failure and novel strain imaging techniques: quantification of myocardial deformation via CMR

CMR is an established imaging modality to investigate the underlying aetiology in CHF patients. In particular, a combination of functional assessment and morphological characterization of the heart allows to distinguish between ischemic and non-ischemic cardiomyopathies according to myocardial fibrosis/scar patterns. Furthermore CMR with T1 mapping and late gadolinium enhancement allows deep myocardial characterization in conditions such as myocarditis, amyloidosis, sarcoidosis and many more (3).

With regard to regional functional assessment, myocardial strain imaging is an advanced imaging technique, which has been established in the past years has an important additional diagnostic and prognostic tool for clinical cardiologists (20). It allows an accurate assessment of regional myocardial function and deformation. In particular, speckle tracking echocardiography is meanwhile playing a relevant role in the clinical daily routine. On the contrary, CMR tissue tracking has not yet being integrated in the clinical routine because of the associated time-consuming post-processing offline analysis (21). In this regard, CMR-FT is a novel technique which has been developed to allow a quick endocardial and epicardial contouring through the detection of the contrast between myocardial tissue and blood pool. Importantly, this technique can be applied to conventional b-SSFP sequences (22), with the aim of speeding up the whole process and facilitating the translation of CMR-FT in the clinical arena. Recently, CMR-FT has been validated against myocardial tagging technique for the assessment of regional myocardial motion in humans (23,24).

Inter- and intra-observer reproducibility are essential in facilitating the abovementioned translational process, and several clinical studies have been published showing excellent results at different field strength MRI scanners (21,25). However, reference datasets and reproducibility data need to be acquired in the preclinical setting as well, especially considering the increasing popularity of CMR in animal research (26). In particular, large animals, such as Landrace pigs, are highly suited to investigate myocardial function under various pharmacological interventions given a cardiac anatomy and physiology closely resembling the one of humans. Current recommendation of the international regulatory authorities underline the importance of large animal model in the process of bench to bedside transition of novel medical compounds. The European Medicines Agency (EMA) guidelines have implemented the Directive 2010/63/ EU, with the result of promoting the utilization of non-rodent species, such as pig and sheep, while discouraging the use of non-human primates in

biomedical research. For example, toxicity studies should be performed in non-human primates merely in case they are the only relevant species which shows a high cross-species reactivity in non-human primates and humans, such as for monoclonal antibodies. Finally, recent comparative data on quantitative myocardial proteomics have shown a high similarity of pig myocardium to humans with regard to in vitro metabolic profile (27).

Aim 2 of this work therefore refers to the reproducibility of LV deformation assessed via CMR-derived global strain indices under various inotropic states in Landrace pigs. Aim 3 investigates the impact of indexing CMR-derived LV strain parameters for indirect measures of afterload on their correlation with invasive hemodynamic indices in experimental acute heart failure.

1.4 Chronic heart failure: future and emerging nanotechnologies.

Despite continuous development in the therapeutic field and the implementation of the four therapeutic pillars (beta-blockers, ACE inhibitors/ARNI, Angiotensin-receptor blockers, sodium-glucose cotransporter inhibitors), mortality in HF is still unacceptably high (3). A great urge is claimed for novel therapeutic compounds for the treatment of HF, which has been recently paralleled by an intensive exploration of cutting-edge nano-formulations capable of safe and efficient drug delivery. To date, no nanotechnology-based clinical product in the cardiovascular field, has been approved by the FDA or EMA. The most prominent contributions in regards of nanocarriers before the coronavirus disease 2019 (COVID-19) pandemic was related to the field of clinical oncology, and played a major role in the clinical trials for novel treatments in cancer patients (28). During the COVID-19 pandemic however, two COVID-19 vaccines, mRNA-1273 and BNT162b21, using lipid nanoparticles (NPs) to deliver antigen mRNA were authorized by EMA and by the U.S. Food and Drug Administration (FDA) (29).

Thus far, a collection of first-generation nanoparticles (NPs) has been approved by the FDA for clinical use, while other liposomal- and polymer-drug conjugates nanocarriers are currently under clinic and preclinic development. Among these, poly(lactide)-Poly(ethylene glycol) NP (PEGylated PLGA NP - BIND-014 BIND Biosci), synthetic NP (vaccine, Selecta Biosciences), liposome (SGT-53 – SynerGene), Poly(ethylene glycol)-lipid NP (Alnylam), Cyclodextrin-PEG NPs (CALAA-01, Calando Pharma, NCT00689065), Stable Nucleic Acid Lipid Particle (SNALP) polyethyleneglycol (PEG)-conjugated liposomes (TKM-ApoB, Tekmira and ALN-PCS, Alnylam). However, while relatively simple to produce and with an achieved level of safety and tolerance, such NPs are generally characterized by an uncontrolled drug release in the bloodstream, poor encapsulation efficacy, and poor stability during storage of liposomes. Additionally, a drastic lack of active targeting challenges the pharmacological specificity required for the achievement of an effective therapy without the adverse reactions of local or systemic toxicity towards unwanted targets.

NPs have also been used for the treatment of pulmonary diseases (e.g. ARIKACE, Pulmaquin). However, inhalation of such NPs might find substantial limitations if aimed for a systemic delivery through translocation across the air-blood-barrier. In line with this, no data of inhaling NPs for the treatment of cardiac conditions have been reported so far. Furthermore, no studies addressing the possibility of using inhaled NPs for the treatment of cardiac conditions have been shown. In addition, despite of great interest in the direct treatment of the diseased heart, the current cardiovascular

trials remain limited towards the monitoring, detection, and treatment of atherosclerosis (e.g. BLAST, NANOM, Nano-Athero), occurring in the vasculature (30).

In the past years, Calcium Phosphate (CaP)-NPs have been developed as non-viral vectors for gene-transfection and drug delivery (31-33), being defined as safe by the Food and Drug Administration (FDA), because of their natural occurrence and biological role in several body-tissues (34). Within the European Union-funded CUPIDO (Cardiac Ultrafficient NanoParticles for Inhalation of Drug prOducts) project, we have developed and tested innovative and unconventional self-assembling CaP-NPs (35). While the concept of biocompatible nanoparticles as drug carrier is already spread in the literature, inhalation of nanoparticles as a “Trojan horse” to deliver compounds to the diseased heart is a novel concept.

In this project, the CaP-NPs were loaded with a therapeutic peptide, the so called mimetic peptide (MP). MP was shown to restore calcium cycling and thus proper cardiomyocyte function through targeting of the L-type Calcium-Channel (LTCC) Cavβ2 cytosolic chaperone (36,37), providing a potential novel tool for HF treatment. Aim 4 of this work refers to the proof of concept of the safety and efficacy of the inhalable formulation based on MP loaded NPs (CaP-MP) in experimental heart failure.

1.5 Acute heart failure and mechanical circulatory support

Cardiogenic shock (CS) is a life-threatening low-cardiac-output state leading to end-organ hypo-perfusion and hypoxia (38). It owns a very high in-hospital mortality notwithstanding current therapeutic advances (39-42). There are many myocardial insults behind this hyper-acute condition, ranging from myocardial ischemia following ST-elevation myocardial infarction to fulminant myocarditis. In each case, a causative diagnosis is essential for timely treatment. The 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure recommend considering mechanical circulatory support (MCS) in patients with cardiogenic shock to improve hypoperfusion and organ dysfunction (3). In the past decades, several MCS devices with peculiar hemodynamic characteristics (43) have been developed beside the classical extracorporeal life support (ECLS) (44), which creates an extremely high afterload and it is detrimental for LV contractility and dimensions (45). In line with this, several modalities of temporarily mechanical ventricular support (MVS) for LV unloading as well as LV decompression under ECLS were investigated (46), including the intra-aortic balloon pump (IABP) (47), the percutaneous transaortic pulsatile assist device iVAC2® (48) and, in recent years, the Impella family of devices (2.5, CP, 5.0 and 5.5, Abiomed, Danvers, Massachusetts) (49). The underlying mode of action (pulsatile vs non-pulsatile) and the amount of liters pumped per minute (e.g. IABP 0.5, iVAC 2L, Impella 2.5 up to 5.5 l/min) differ between different MVS.

The Impella devices are transcutaneously implanted pumps producing an anterograde, axial blood flow and therefore beneficially unloading the LV (45,50). In recent years, Impella systems have been applied in several cohorts of CS patients, especially those affected by an acute myocardial infarction (AMICS) or by an acute fulminant myocarditis, with the aim of hemodynamic stabilization, early catecholamine weaning and, to some extent, ventricular decompression or unloading. While in the first cohort (AMICS), a MVS is mostly applied over days, in the latter can be performed in a prolonged fashion over weeks, according to the so called PROPELLA concept (51), allowing myocardial recovery in awake and mobilized patients. However, the literature on weaning from temporary LV mechanical support systems is scarce, as the established weaning algorithms mostly refer to LV assist device (LVAD) patients (52). Weaning from MVS is therefore mostly performed in the absence of established algorithms (53-55), and strongly relies on the individual experience of the single centres involved. Furthermore, given the increasingly popularity in CS management of a combination of ECLS with LV unloading via Impella, the so called ECMELLA strategy (51,56), novel

weaning algorithms are essential for a standardized clinical approach. Currently, a de-escalation of Impella® in ECMELLA patients is usually attempted after stabilization of the respiratory function as well as after complete catecholamine weaning of the patient, as observed during stepwise reduction of the ECLS blood flow.

In Summary, a clear weaning strategy after ECLS removal in ECMELLA patents or in PROPELLA patients is therefore still lacking. Aim 5 of this study is related to the weaning algorithm established at our centre for hemodynamic-driven, structured weaning of CS patients undergoing MVS with Impella.

2. Aims

Main focus of this dissertation is the investigation and further characterization of invasive and non-invasive indices of LV function and cardiovascular hemodynamics both in preclinical (Sus scrofa) as well as clinical HF. Following topics were addressed in the current work:

1. The correlation between LV CPO, as clinically relevant index of external LV work, and the conductance catheter-derived LV SW over a wide range of contractility states in experimental acute heart failure in Landrace pigs.

2. The reproducibility and reference values of global LV strain indices assessed via a novel CMR-FT analysis under various inotropic states in Landrace pigs.

3. The impact of indexing CMR-derived LV strain parameters for indirect measures of afterload on their correlation with invasive hemodynamic indices in experimental acute heart failure in Landrace pigs.

4. The impact on LV contractility of a novel, inhalable, cardiac-specific nanocarrier delivering a LTCC-modulating peptide in experimental CHF in mice and Landrace pigs.

5. The assessment of LV hemodynamics with non-invasive and invasive techniques for a structured weaning of CS patients undergoing MVS with the Impella device.

3. Original articles

The following original articles have been published in peer-reviewed journals over the past 3 years. Main conceptual core of this work is heart failure and its hemodynamic characterization. The paragraphs 3.1 to 3.4 present articles related to the experimental, preclinical work performed by the author and colleagues in the large animal laboratory and include several datasets from acute and chronic heart failure models. Furthermore, while the first 3 paragraphs relate to the characterization of LV hemodynamics, invasively as well as non-invasively, paragraph 3.4 introduces a potential novel therapy for chronic HF based on a biocompatible self-assembling nanocarrier. Finally, paragraph 3.5 is related to clinical data collected in AHF patients undergoing MCS and thus constitutes a glimpse of translational hemodynamics.

With regard to the preclinical part, the experimental work has been performed in Landrace pigs. The first original article (3.1) explores the value of CPO, an important though underused clinical index of LV hydraulic power, in mirroring LV stroke work over time in experimental AHF induced by different type of cardiac insults, ranging from myocardial infarction to sepsis. A better physiological understanding of the role of CPO over a wide range of inotropic states is essential for supporting its use in the intensive care unit (ICU)setting and this article tries to answers some of the open questions related to the physiological meaning of CPO.

In the second original article (3.2) we report on the reproducibility of LV deformation assessed via CMR-derived global strain indices under various inotropic states. Dobutamine-induced LV hypercontractility as well as verapamil-induced LV hypocontractility were induced in healthy, intubated Landrace pigs during CMR scan. Data on inter- and intra-reproducibility of LV strain measurements were collected. Based on the LV strain data, a sample size calculation was performed, in order to identify the minimum number of animals needed for testing relative changes of LV contractility. These data are intended to set the reference strain values for future preclinical trials, in accordance to the 3R principle of reduction of number of animal experiments in biomedical research.

In the third original article (3.3), we describe the impact of indexing CMR-derived LV strain parameters for indirect measures of afterload on their correlation with invasive hemodynamic indices in the same experimental setting as described in paragraph 3.2, i.e. LV hyper and

hypocontractility. In particular, strain indices describe LV deformation over the cardiac cycle, while not taking into account the role of LV and systemic pressures. Strain measurements are therefore susceptible to changes in loading conditions, which may therefore impact on their reproducibility and physiological meaning. Paragraph 3.3 shows that simple approximation of afterload change over time improves the accuracy of LV strain indices in reflecting classical hemodynamic measures, such as cardiac index (CI), power and end-systolic elastance (Ees).

The last experimental paragraph 3.4 describes a novel inhalable cardiac specific nanocarrier delivering a LTCC-modulating peptide exerting a positive inotropic effect in experimental chronic heart failure in mice. The author and colleagues performed a further proof of concept of the myocardial delivery of the abovementioned compound after inhalation in Landrace pigs, showing myocardial accumulation of a marker peptide over time.

Finally, in paragraph 3.5, given the lack of data in literature on weaning from temporary mechanical circulatory support devices such as the novel Impella pumps, we describe the weaning protocol developed in our centre over the past years and its outcome. The acronym “TIDE” simply describes the 4 steps of our weaning protocol, namely Transthoracic Echocardiographic examination during unloading, Impella pump rate reduction, Dobutamine stress echocardiography and Exercise testing during catheterization, in order to assess hemodynamic characteristics of Impella unloaded patients and guide further steps of their therapeutic strategy.

3.1 Cardiac power output accurately reflects external cardiac work over a wide range of inotropic states in pigs

In this experimental study, we were able to show an excellent correlation between LV CPO and LV stroke work as invasively assessed by conductance catheter after inducing acute heart failure at several body temperatures. With these data we would like to underline the pathophysiological relevance of CPO for hemodynamic monitoring of ICU patients.

The text below is the abstract of the publication: Abawi D, Faragli A, Schwarzl M, Manninger M, Zweiker D, Kresoja KP, Verderber J, Zirngast B, Maechler H, Steendijk P, Pieske B, Post H, Alogna A, Cardiac power output accurately reflects external cardiac work over a wide range of inotropic states in pigs, BMC Cardiovascular Disorders. 2019 Oct 15;19(1):217. DOI: https://doi.org/10.1186/s12872-019-1212-2 (57)

Background: Cardiac power output (CPO), derived from the product of cardiac output and mean aortic pressure, is an important yet underexploited parameter for hemodynamic monitoring of critically ill patients in the intensivecare unit (ICU). The conductance catheter-derived pressure-volume loop area reflects left ventricular stroke work (LV SW). Dividing LV SW by time, a measure of LV SW min− 1 is obtained sharing the same unit as CPO (W). We aimed to validate CPO as a marker of LV SW min− 1 under various inotropic states.

Methods: We retrospectively analysed data obtained from experimental studies of the hemodynamic impact of mild hypothermia and hyperthermia on acute heart failure. Fifty-nine anaesthetized and mechanically ventilated closed-chest Landrace pigs (68 ± 1 kg) were instrumented with Swan-Ganz and LV pressure-volume catheters. Data were obtained at body temperatures of 33.0 °C, 38.0 °C and 40.5 °C; before and after: resuscitation, myocardial infarction, endotoxemia, sevoflurane-induced myocardial depression and beta-adrenergic stimulation. We plotted LVSW min− 1 against CPO by linear regression analysis, as well as against the following classical indices of LV function and work: LV ejection fraction (LV EF), rate-pressure product (RPP), triple product (TP), LV maximum pressure (LVPmax) and maximal rate of rise of LVP (LV dP/dtmax).

Results: CPO showed the best correlation with LV SW min− 1 ($r^2 = 0.89$; $p < 0.05$) while LV EF did not correlate at all ($r^2 = 0.01$; $p = 0.259$). Further parameters correlated moderately with LV SW min− 1 (LVPmax $r^2 = 0.47$, RPP $r^2 = 0.67$; and TP $r^2 = 0.54$). LV dP/dtmax correlated worst with LV SW min− 1 ($r^2 = 0.28$).

Conclusion: CPO reflects external cardiac work over a wide range of inotropic states. These data further support the use of CPO to monitor inotropic interventions in the ICU.

3.2 Cardiovascular magnetic resonance feature tracking in pigs: a reproducibility and sample size calculation study.

In this study, we were able to show a good to excellent inter- and intra-observer reproducibility of CMR-FT derived LV strain indices in pigs under various inotropic states. In particular, GLS was outperforming global circumferential (GCS) and radial strain (GRS). Accordingly, a sample size calculation showed a low number of animals (n<10) needed to predict a 10 to 15% GLS change with this experimental setup. In summary, these data represent a relevant contribution to the CMR literature, establishing reference values for future pharmacological investigation in Landrace pigs.

The text below is the abstract of the publication: Faragli A, Tanacli R, Kolp C, Lapinskas T, Stehning C, Schnackenburg B, Lo Muzio FP, Perna S, Pieske B, Nagel E, Post H, Kelle S*, Alogna A*, Cardiovascular magnetic resonance feature tracking in pigs: a reproducibility and sample size calculation study. International Journal of Cardiovascular Imaging, 2020 Apr;36(4):703-712. DOI: https://doi.org/10.1007/s10554-020-01767-y (58)

Cardiovascular magnetic resonance feature tracking (CMR-FT) is a novel technique for non-invasive assessment of myocardial motion and deformation. Although CMR-FT is standardized in humans, literature on comparative analysis from animal models is scarce. In this study, we measured the reproducibility of global strain under various inotropic states and the sample size needed to test its relative changes in pigs. Ten anesthetized healthy Landrace pigs were investigated. After baseline (BL), two further steps were performed: (I) dobutamine-induced hyper-contractility (Dob) and (II) verapamil-induced hypocontractility (Ver). Global longitudinal (GLS), circumferential (GCS) and radial strain (GRS) were assessed. This study shows a good to excellent inter- and intra-observer reproducibility of CMR-FT in pigs under various inotropic states. The highest inter-observer reproducibility was observed for GLS at both BL (ICC 0.88) and Ver (ICC 0.79). According to the sample size calculation for GLS, a small number of animals could be used for future trials.

3.3 Cardiovascular magnetic resonance-derived left ventricular mechanics-strain, cardiac power and end-systolic elastance under various inotropic states in swine.

This study addresses the question of the impact of hemodynamic loading, in particular afterload, on LV strain indices. We could show that indexing global strain parameters for indirect measures of afterload, such as mean aortic pressure or wall stress, improves the correlation of GLS, GCS and GRS with classical indices such as CI, CPO and Ees. In addition, GLS indexed for wall stress reflected LV contractility as good as the clinically widespread LV EF. These data support further investigations in the field of CMR-FT-strain imaging, as a quick and promising tool to characterize LV hemodynamics in patients with varying degrees of LV dysfunction.

The text below is the abstract of the publication: Faragli A, Tanacli R, Kolp C, Abawi D, Lapinskas T, Stehning C, Schnackenburg B, Lo Muzio FP, Fassina L, Pieske B, Nagel E, Post H, Kelle S, Alogna A, Cardiovascular magnetic resonance-derived left ventricular mechanics-strain, cardiac power and end-systolic elastance under various inotropic states in swine. Journal of Cardiovascular Magnetic Resonance. 2020 Nov 30;22(1):79. DOI: https://doi.org/10.1186/s12968-020-00679-z (59)

Background: Cardiovascular magnetic resonance (CMR) strain imaging is an established technique to quantify myocardial deformation. However, to what extent left ventricular (LV) systolic strain, and therefore LV mechanics, reflects classical hemodynamic parameters under various inotropic states is still not completely clear. Therefore, the aim of this study was to investigate the correlation of LV global strain parameters measured via CMR feature tracking (CMR-FT, based on conventional cine balanced steady state free precession (bSSFP) images) with hemodynamic parameters such as cardiac index (CI), cardiac power output (CPO) and end-systolic elastance (Ees) under various inotropic states.

Methods: Ten anaesthetized, healthy Landrace swine were acutely instrumented closed-chest and transported to the CMR facility for measurements. After baseline measurements, two steps were performed: (1) dobutamine-stress (Dobutamine) and (2) verapamil-induced cardiovascular depression (Verapamil). During each protocol, CMR images were acquired in the short axisand apical 2Ch, 3Ch and 4Ch views. MEDIS software was utilized to analyze global longitudinal (GLS), global circumferential (GCS), and global radial strain (GRS).

Results: Dobutamine significantly increased heart rate, CI, CPO and Ees, while Verapamil decreased them. Absolute values of GLS, GCS and GRS accordingly increased during Dobutamine infusion, while

GLS and GCS decreased during Verapamil. Linear regression analysis showed a moderate correlation between GLS, GCS and LV hemodynamic parameters, while GRS correlated poorly. Indexing global strain parameters for indirect measures of afterload, such as mean aortic pressure or wall stress, significantly improved these correlations, with GLS indexed for wall stress reflecting LV contractility as the clinically widespread LV ejection fraction.

Conclusion: GLS and GCS correlate accordingly with LV hemodynamics under various inotropic states in swine. Indexing strain parameters for indirect measures of afterload substantially improves this correlation, with GLS being as good as LV ejection fraction in reflecting LV contractility. CMR-FT-strain imaging may be a quick and promising tool to characterize LV hemodynamics in patients with varying degrees of LV dysfunction.

3.4 Inhalation of peptide-loaded nanoparticles improves heart failure.

In this study, we envision a novel therapeutic concept for HF patients, based on the inhalation of a newly designed small molecule beneficially impacting on cardiac contractility. The role of peptides as potentially disruptive disease modifying molecules for the treatment of cardiovascular diseases has emerged in the past years. We previously designed a novel small peptide (MP), as a novel therapeutic tool for the improvement of cardiac conditions correlated with alterations in LTCC levels and function. However, chronic administration of a peptide is obviously limited by its quick systemic degradation and therefore short half-life. In the current work, we could show that the inhalation of CaPs loaded with MP improves cardiac contractility in a rodent model of diabetic cardiomyopathy. Furthermore, we successfully performed a scale-up of this concept in large animal, and we here present a first feasibility, proof-of concept study investigating the myocardial accumulation of a marker peptide after inhalation of CaPs in Landrace pigs. Overall, this preclinical study addresses a novel therapeutic concept for HF patients, and further developments towards a first-in-human application are planned.

The text below is the abstract of the publication: Miragoli M, Ceriotti P, Iafisco M, Vacchiano M, Salvarani N, Alogna A, Carullo P, Ramirez-Rodríguez GB, Patrício T, Esposti LD, Rossi F, Ravanetti F, Pinelli S, Alinovi R, Erreni M, Rossi S, Condorelli G, Post H, Tampieri A, Catalucci D. Inhalation of peptide-loaded nanoparticles improves heart failure. Sci Transl Med. 2018 Jan 17;10(424):eaan6205. doi: https://doi.org/10.1126/scitranslmed.aan6205. (37)

Peptides are highly selective and efficacious for the treatment of cardiovascular and other diseases. However, it is currently not possible to administer peptides for cardiac-targeting therapy via a noninvasive procedure, thus representing scientific and technological challenges. We demonstrate that inhalation of small (<50 nm in diameter) biocompatible and biodegradable calcium phosphate nanoparticles (CaPs) allows for rapid translocation of CaPs from the pulmonary tree to the bloodstream and to the myocardium, where their cargo is quickly released. Treatment of a rodent model of diabetic cardiomyopathy by inhalation of CaPs loaded with a therapeutic mimetic peptide that we previously demonstrated to improve myocardial contraction resulted in restoration of cardiac function. Translation to a porcine large animal model provides evidence that inhalation of a peptide-loaded CaP formulation is an effective method of targeted administration to the heart.

Together, these results demonstrate that inhalation of biocompatible tailored peptide nanocarriers represents a pioneering approach for the pharmacological treatment of heart failure.

3.5 The "TIDE"-Algorithm for the Weaning of Patients with Cardiogenic Shock and Temporarily Mechanical Left Ventricular Support With Impella Devices. A Cardiovascular Physiology-Based Approach.

In this work, we propose a novel cardiovascular physiology-based weaning algorithm for patients with CS undergoing MVS with Impella. This algorithm, described by the acronym TIDE, aims at a better hemodynamic stratification of patients, based on the characterization of the extent and sustainment of LV unloading reached during hospitalization. Prospective studies are needed to validate the algorithm.

The text below is the abstract of the publication: Tschöpe C, Spillmann F, Potapov E, Faragli A, Rapis K, Nelki V, Post H, Schmidt G, Alogna A, The "TIDE"-Algorithm for the Weaning of Patients With Cardiogenic Shock and Temporarily Mechanical Left Ventricular Support With Impella Devices. A Cardiovascular Physiology-Based Approach. Frontiers in Cardiovascular Medicine, 2021 Feb 19;8:563484. doi: https://doi.org/10.3389/fcvm.2021.563484 (46)

Objectives: *Mechanical circulatory support (MCS) is often required to stabilize therapy-refractory cardiogenic shock patients. Left ventricular (LV) unloading by mechanical ventricular support (MVS) via percutaneous devices, such as with Impella® axial pumps, alone or in combination with extracorporeal life support (ECLS, ECMELLA approach), has emerged as a potential clinical breakthrough in the field. While the weaning from MCS is essentially based on the evaluation of circulatory stability of patients, weaning from MVS holds a higher complexity, being dependent on bi-ventricular function and its adaption to load. As a result of this, weaning from MVS is mostly performed in the absence of established algorithms. MVS via Impella is applied in several cardiogenic shock etiologies, such as acute myocardial infarction (support over days) or acute fulminant myocarditis (prolonged support over weeks, PROPELLA). The time point of weaning from Impella in these cohorts of patients remains unclear. We here propose a novel cardiovascular physiology-based weaning algorithm for MVS.*

Methods: *The proposed algorithm is based on the experience gathered at our center undergoing an Impella weaning between 2017 and 2020. Before undertaking a weaning process, patients must had been ECMO-free, afebrile, and euvolemic, with hemodynamic stability guaranteed in the absence of any inotropic support. The algorithm consists of 4 steps according to the acronym TIDE: (i) Transthoracic echocardiography under full Impella-unloading; (ii) Impella rate reduction in single 8–24 h-steps according to patients hemodynamics (blood pressure, heart rate, and ScVO2), including a*

daily echocardiographic assessment at minimal flow (P2); (iii) Dobutamine stress-echocardiography; (iv) Right heart catheterization at rest and during Exercise-testing via handgrip. We here present clinical and hemodynamic data (including LV conductance data) from paradigmatic weaning protocols of awake patients admitted to our intensive care unit with cardiogenic shock. We discuss the clinical consequences of the TIDE algorithm, leading to either a bridge-to-recovery, or to a bridge-to-permanent LV assist device (LVAD) and/or transplantation. With this protocol we were able to wean 74.2% of the investigated patients successfully. 25.8% showed a permanent weaning failure and became LVAD candidates.

Conclusions: *The proposed novel cardiovascular physiology-based weaning algorithm is based on the characterization of the extent and sustainment of LV unloading reached during hospitalization in patients with cardiogenic shock undergoing MVS with Impella in our center. Prospective studies are needed to validate the algorithm.*

4. Discussion

4.1 Invasive and non invasive assessment of cardiac work : clinical implications

Invasive and non-invasive parameters reflecting cardiac work are still underexploited in the clinical arena (15). In the current work (57), CPO obtained via a pulmonary artery catheter (PAC) showed an excellent correlation with the gold standard, conductance-derived LV SW in experimental conditions resembling critically-ill patients, including resuscitation after ventricular fibrillation, acute coronary no-reflow infarction and endotoxemia by LPS-infusion among others. Of note, CPO reflected LV SW with a higher accuracy than several pressure and pressure-derived indices of cardiac workload, such as the rate pressure product (RPP) and the triple product (TP).

CPO describes the ability of the heart to create pressure and flow, i.e. it defines the heart as a hydraulic pump (60). The power generated by the heart has two distinct components: the mean external power, which allows to generate a steady (non-pulsatile) flow (well approximated by CPO), and the pulsatile power, producing the pulsatile component of the flow. The sum of both is the total external power (well represented on a beat to beat basis by LV SW) of the ventricle (61). The pulsatile power is under physiological conditions negligible, making up for 10% of the total external power. It plays a more relevant role in pathological conditions, as well as in the pulmonary circulation (62,63). Even if CPO in theory merely represents the mean power, its assessment was shown to have strong prognostic implications, being the best hemodynamic correlate of intra-hospital mortality in patients with cardiogenic shock (19). Nevertheless, the role of CPO into the clinical ICU routine is still unclear, probably because of the neutral effects of pulmonary arterial catheter (PAC) monitoring on survival (64) and the resulting diminished popularity of PAC monitoring in the ICU. The experimental data presented in our work underline the pathophysiological relevance of CPO as a reliable parameter to monitor hemodynamic changes under a broad spectrum of inotropic states. Furthermore, CPO reflected LV SW with a higher accuracy than classical indices of myocardial oxygen consumption, such as the RPP and the TP, both regarded in literature as clinically relevant indirect measures of cardiac work (65,66).

Recently, a novel non-invasive method to assess myocardial work is widening the potential application of indices such as CPO in the clinical, outpatient routine. In fact, Russel and colleagues were able to obtain pressure-strain-loops by speckle-tracking echocardiography (67). This method

is based on a simple LV GLS, as estimated by standard processing of the apical two-, three-, and four-chamber views. An algorithm to estimate left ventricular pressure (LVP) from non-invasively measured systemic arterial pressure has been developed and it is part of the commercially available plug in for pressure-strain analysis. The normalization of left ventricular pressure curves was derived by interpolating invasive LVP measurements from patients in different inotropic conditions (67). Global values of myocardial work are finally derived by averaging all segmental myocardial work values. In particular, constructive and wasted work, as well as myocardial work efficiency resemble the invasively measured SW, PE and cardiac work efficiency and are therefore becoming increasingly popular. In the past years, several investigations have been initiated with regard to cardiovascular patients' cohorts: CRT recipients, patients with ischemic cardiac disease, mitral valve repair, HFrEF, HFpEF or HFpEF-like syndromes (hypertrophic cardiomyopathy, cardiac amyloidosis). We have recently reviewed the implications of this fast-growing field of clinical research, while underlying the potential of pressure-strain loops for non-invasive estimation of myocardial work in both acute and chronic HF patients. The mechanistic data presented in this work therefore address once again the need of further investigation of the role of myocardial work for monitoring cardiac function in both AHF and CHF patients (15). In particular, further work should clarify the prognostic implication of myocardial work assessment over the course of disease in CHF patients.

4.2 Reproducibility of cardiovascular magnetic resonance feature tracking in pigs.

Classification of patients according to their LV EF plays a major role in HF diagnosis and treatment (3). However, LV EF is highly load-dependent and suffers from significant intraobserver and interobserver variability (68). In addition, LV EF falls too short in describing regional myocardial function. Assessing regional myocardial function via myocardial strain has become increasingly popular, because of the relevant amount of added information which can be extrapolated from its use. Speckle tracking echocardiography allows to assess myocardial strain in three spatial directions (longitudinal, radial and circumferential), while being as independent as possible from the angle of the ultrasound beam. Radial and circumferential strain seem to be less reproducible than longitudinal strain, which is probably the most frequent type of strain used to characterise LV systolic function in clinical practice (69). Echocardiographic imaging of LV deformation via myocardial strain has now moved from being a research tool to having an established role in HF management. In particular, myocardial strain provides information on risk prediction, diagnosis, assessment of treatment response, and follow-up of HF patients (70).

On the contrary, CMR tissue tracking has still limited clinical applications because of the associated time-consuming post-processing offline analysis (21). CMR-FT is a novel technique developed to overcome this issue and allow a quick integration of myocardial strain in the clinical routine. This technique focuses on contouring of myocardial layers on the basis of the natural contrast between myocardium and blood pool (22,71). Several investigations assessing inter and intra-observer reproducibility for different CMR-FT derived indices and at different field strength MRI scanners (25,72,73) have been performed. However, there is a lack of standardization, reproducibility studies and of reference databases in the preclinical setting. In fact, while the analysis on reproducibility of new imaging techniques represents a routine standard for clinical studies, literature on comparative analysis from animal studies is scarce. In line with this, we published a study reporting on the excellent reproducibility of myocardial strain via CMR-FT in a rodent model, therefore contributing to a recent guideline on imaging for animal research (74,75). Imaging studies in rodents have major drawbacks related to different species-related anatomical and physiological characteristics. Non-rodent imaging is becoming increasingly popular, while small animals are hardly comparable to humans, in particular regarding the assessment of myocardial function. To the best of our knowledge, only one study in the literature has investigated the

reproducibility of myocardial deformation analysis in large animals, namely in nonhuman primates (76) and no literature can be found on Landrace pigs.

In this work (58), we analysed the reproducibility of global strain indices under various inotropic states in Landrace pigs, providing a further evidence of the reliability and reproducibility of global strain assessed by CMR-FT. In detail, GLS showed higher reproducibility than GCS and GRS, in line with what reported on speckle tracking (69). In addition, the sample size calculation based on this dataset showed a low number of animals (n<10) needed to predict a 10 to 15% GLS change with this experimental setup. These data are therefore a relevant contribution to the literature with reference values for CMR investigation in Landrace pigs, potentially allowing a reduction of the number of animal experiments required for novel investigations, in accordance with the 3R principle. Combining sample size calculation, already required form the regulatory authorities in the European countries for study approval, with open-access databases on medical imaging datasets, could booster large animal models as a platform tool to test the effect of novel compounds.

4.3 Indexing strain parameters for indirect measures of afterload: correlation with invasive hemodynamic parameters.

As discussed in the previous paragraph, CMR myocardial deformation assessment is quickly progressing from a mere research tool towards routine clinical application. However, the correlation between LV systolic strain, and therefore LV mechanics, and classical hemodynamic parameters is still unclear. The role of loading conditions and its impact on the reproducibility of strain is surely an important issue to be addressed. In this study (59) of 10 anesthetized healthy female Landrace pigs, left ventricular global strain parameters were measured using CMR-FT, and correlated with simultaneous invasive hemodynamic measurements under various inotropic states (baseline, and after administration of dobutamine and verapamil). Global longitudinal and circumferential strains were found to correlate with CI, CPO and Ees. Furthermore, the correlations was stronger when strain parameters were indexed for indirect measures of afterload.

FT-strain is based on simple steady-state free precession sequences (24,77-80), and therefore allows a quick assessment of LV deformation essential for clinical translation. Furthermore, we indexed GLS, GCS and GRS for indirect measures of afterload, such as the invasively measured mAOP as well as the meridional wall stress, as already described by Rhea et al. and Reichek et al (81,82), in order to take into account load-dependency of LV deformation imaging. Interestingly, the afterload corrected strain parameters showed an improved correlation with LV hemodynamics. This concept is in line with other reports in literature emphasizing the role of pressure adjustment of myocardial strain. Yingchoncharoen et al. demonstrated the advantage of adjusting strain indices for blood pressure in patients with large deviations of systolic blood pressure from the normal-reference value (83). These results were confirmed by Weiner et al., investigating the impact of isometric handgrip testing on left ventricular twist mechanics and the influence of blood pressure on GLS (84). Finally, Rhea at al. showed an added prognostic value of pressure-adjusted longitudinal strain in predicting cardiac events and mortality(82).

We therefore conclude that afterload correction of strain may add accuracy to the evaluation of the mechanical and contractile function of the heart without being time-consuming. We envision a promising role for CMR-FT LV strain investigation of chronic HF patients, with particular regard to HFpEF patients, given previous studies showing a diagnostic and prognostic impact of strain measurements (85).

4.4 Pharmacological targeting of the heart: a novel nanocarrier and future therapeutical implications.

In the past decades, the field of nanomedicine has displayed a quick development as a potential game-changer in several clinical fields. Novel pharmaceutical technologies and materials have boosted the field of NPs as carriers for a precision medicine, conceptually based on tissue-specific targeting and controlled release of small molecules for diagnostic or therapeutic purposes. While the first generation of NPs, such as lipid-based NPs, were mostly designed to improve pharmacokinetic proprieties of existing drugs without compromising on patients' safety, in the past years our knowledge on NP-loaded drugs and their pharmacodynamics and tissue distribution has exponentially increased. We have previously summarized (30) the most notable, common features of the novel NP-loaded drugs, including i) protection from systemic degradation, ii) reduced toxicity and immunogenicity, iii) ameliorated pharmacokinetics with increased half-life, and iv) increased bioavailability and precise biodistribution (30). These features are heavily influenced by the route of drug administration, traditionally oral, intravenous or subcutaneous, and we propose here inhalation as a suitable route for NPs' delivery to the heart (30).

In the study here presented (37), NPs have been designed as a "trojan horse" for direct cardiac release of a novel therapeutic compound upon inhalation. While the detrimental effect of the so called lung-to-heart phenomenon on cardiovascular health has been clearly demonstrated in the field of air pollution research, including ultrafine (<0.1 µm) particulate (86), the therapeutic potential of the same route has neve been investigated. We therefore set out to exploite this administration route generating bioinspired and self-assembling CaP NPs (35). CaP NPs are highly biocompatible and biodegredable, and possess several important features important for clinical application, above all the capability of crossing biological barriers, such as the one at the pulmonary level (30,37). This opens up a novel scenario in cardiovascular disease, given that the few inhalable nano-formulations developed in the past decades have been designed for the treatment of pulmonary diseases (87,88) (89). We here demonstrate that inhalation of bioresorbable and negatively charged CaP nanocarriers (35) allows for safe targeting to and treatment of the heart.

Our data show that inhalation of CaP NPs encapsulating MP, a small peptide modulating LTCC, restores LV contractility in a murine model of streptozotocin-induced diabetic cardiomyopathy. The in vivo results were further mirrored by improvement of molecular and

cellular defects associated with diabetic cardiomyopathy, including restoration of LTCC protein levels as well as recovery of LTCC currents in isolated cardiomyocytes from CaP-MP-treated mice, in line with previous reports by Rusconi and colleagues (36).

Furthermore, the non-viral vectorial delivery (CaP-NP) to the heart was effective not only in mice, but also in anesthetized and intubated Landrace pig. The safety profile and the pharmacokinetic of the developed CaPNPs were excellent, with no acute safety concerns as well as stable and reproducible myocardial cargo release over 5 hours after inhalation. A translational implication of these data is the low amount of required therapeutic compound for myocardial delivery, potentially minimizing systemic spill-over and side effects when compared to systemic delivery. Potential limitations of the clinical translation of this approach are related to pathological conditions affecting the lungs such as chronic obstructive pulmonary or pulmonary congestion, impacting on CaP persistence and translocation toward the pulmonary bloodstream.

In conclusion, this work investigates a novel approach for specific cardiac targeting based on a biocompatible nanocarrier for the treatment of HF with therapeutic peptides. A wider use of CaPs as a non-viral vector for DNA/RNA based therapies with different release kinetics (therapeutic polypharmacy) will be further investigated.

4.5 The "TIDE"-Algorithm for the Weaning of Patients With Cardiogenic Shock and Temporarily Mechanical Left Ventricular Support With Impella Devices. A Cardiovascular Physiology-Based Approach.

In this work (46), we report our experience with Impella-mediated LV venting in cardiogenic shock due to acute fulminant myocarditis. We propose a novel cardiovascular physiology-based weaning algorithm (TIDE), discussing potential outcomes of the algorithm, namely bridge-to-recovery or bridge-to-permanent LVAD and/or transplantation. In particular, we focus on LV unloading by percutaneous transvalvular axial-flow devices, such as the Impella family of devices (2.5, CP, and 5+ (49)) because they recently emerged as a potential ground-breaking device therapy when applied in patients with acute fulminant myocarditis as a prolonged support over several weeks in awake, mobilized patients (PROPELLA (51)). The most of the ICUs work in the absence of clear weaning protocols, given the scarcity of literature on this topic. As a matter of fact, the existing data are mainly based on ECLS studies, which are characterized by a shorter duration of mechanical support, ranging from 3 up to 8 days (54,90).

In general, a prerequisite for the beginning of the weaning trial is the reversibility of the aetiology that led to cardiogenic shock, with a fully stabilized hemodynamic condition (91,92), i.e. a baseline mean arterial pressure (MAP) of >60 mmHg in the absence or at low doses of vasoactive agents and a pulsatile arterial waveform maintained for at least 24 hours (92-95). Furthermore, recovery of hepatic function seems to be essential before starting the weaning of patients from ECLS (95), while this does not apply to kidney function (96)).

Once the prerequisites for a weaning trial have been documented, the weaning algorithm proposed in this work (46) can be started. It is based on 4 steps according to the acronym TIDE: (i) Transthoracic echocardiography under full Impella-unloading; (ii) Impella rate reduction in single 8–24 h-steps according to patients hemodynamics (blood pressure, heart rate, and ScVO2), including a daily echocardiographic assessment at minimal flow (P2); (iii) Dobutamine stress-echocardiography; (iv) Right heart catheterization at rest and during Exercise-testing via handgrip (46). Among the four steps, the first two are relevant for all patients, while while step iii and iv should be considered in selected patients with either an unclear contractile reserve (iii) and/or in patients to be bridged towards LVAD implantation or heart transplantation (iv) (46). Of note, 74.2% of the investigated patients was successfully weaned applying the TIDE algorithm, while 25.8% failed

to complete the weaning process, and therefore underwent LVAD/transplantation. These numbers are clearly in line with the most recent ECLS literature (94,95).

The literature focusing on weaning protocols including detailed echocardiographic assessment is scarce. To the best of our knowledge, two ECLS weaning studies have been published and were based on transthoracic (93) and transoesophageal (97) echocardiography. In the first one (93), ECLS support was gradually reduced of about 2/3, then to 1/3 of initial support for 10 -15 minutes and finally to a minimum of 1-1.5l / min. A Blood pressure drop lead to termination of the weaning trial. Minimal LV criteria for a weaning attempt were defined as a LVEF > 20% and an aortic VTI > 10cm. In the second study, the focus of the echocardiographic examination was on biventricular interaction, given the well-known impact of ECLS retrograde flow on LV function and the enhanced interdependence between LV and RV. Furthermore, the focus of ECLS weaning trials has been mostly on preserving hemodynamic stability under minimal mechanical support (46). This differs substantially from the TIDE protocol, where LV contractile reserve evaluation plays a major role for clinical decision-making.

In conclusion, we describe here a novel weaning algorithm for prolonged MVS patients unloaded via an Impella device. The data collected in our centre should be further confirmed in multicentre, randomized clinical trials in order to assess its potential to impact on CS patients.

5. Conclusion

HF is a severe, chronic condition still characterized by unacceptable mortality rates. Gaps in evidence listed within the most recent ESC HF Guidelines 2021 focus on both better phenotyping of HF patients as well as tailored therapeutic approaches to different individuals.

In this work, we investigated i) the role of invasive hemodynamic parameters in reflecting external cardiac work in comparison to their non-invasively assessable counterpart (e.g. LV EF) in experimental heart failure, ii) the reproducibility of CMR-FT for non-invasive assessment of myocardial motion and deformation in Landrace pigs. iii) the potential impact of indexing LV strain parameters for indirect measures of afterload on their prediction of LV contractile function. Furthermore, iv) we developed a novel cardiovascular-physiology-based algorithm for weaning of mechanical circulatory support with Impella in cardiogenic shock patients. Finally, v) we reported on a novel, therapeutic concept based on an inhalable nanotechnology targeting the heart in experimental HF.

In i) we were able to show that LV CPO is an important indicator of LV hydraulic function and accurately predicts external cardiac work under various inotropic states. We therefore concluded that CPO should be used to monitor inotropic interventions in the ICU. Given the prominent role of CPO in the prognostic assessment of patients admitted with cardiogenic shock, as well as the recent advances in the field of non-invasive pressure-strain loops, we suggest to promote clinical study implementing indices of cardiac work in the management of chronic HF patients as well.

In ii) we showed a good to excellent intra and inter-observer reproducibility of CMR-FT in pigs under various inotropic states. The highest inter-observer reproducibility was observed for GLS both in baseline as well as failing conditions. In addition, we calculated the sample size for detecting a GLS relative change as large as the one induced in this animal trial. This will help reduce the number of animals used for future pharmacological trials, in accordance to the 3R (replace, reduce, refine) principle in animal research.

In iii) we showed that LV global strain parameters such as GLS and GCS correlate accordingly with LV hemodynamics (Ees and CPO) under various inotropic states in swine, while indexing strain parameters for indirect measures of afterload substantially improves this correlation. Afterload-

indexed CMR-FT-strain imaging may be a fast tool for in-depth characterization of LV hemodynamics in HF patients.

In iv) we were able to show that the inhalation of biocompatible (CaP) tailored peptide nanocarriers impacting on L-TCC is a pioneering approach for the pharmacological treatment of heart failure, while the translation of the CaP nanocarrier to a porcine large animal model provided evidence that this is an effective method of targeted administration to the heart.

In v) we describe the TIDE (Transthoracic echocardiography, progressive Impella rate reduction, Dobutamine stress-echocardiography; right heart catheterization at rest and during Exercise-testing) algorithm for Impella weaning in cardiogenic shock patients. With this protocol we were able to wean the majority of patients (74.2%) successfully. Prospective studies are needed to validate the algorithm.

6. Literature

1. Conrad N, Judge A, Tran J et al. Temporal trends and patterns in heart failure incidence: a population-based study of 4 million individuals. Lancet 2018;391:572-580.
2. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2018;392:1789-1858.
3. McDonagh TA, Metra M, Adamo M et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. European heart journal 2021;42:3599-3726.
4. Jain CC, Borlaug BA. Hemodynamic assessment in heart failure. Catheter Cardiovasc Interv 2020;95:420-428.
5. Faragli A, Abawi D, Quinn C et al. The role of non-invasive devices for the telemonitoring of heart failure patients. Heart Fail Rev 2021;26:1063-1080.
6. Bozkurt B, Coats AJS, Tsutsui H et al. Universal definition and classification of heart failure: a report of the Heart Failure Society of America, Heart Failure Association of the European Society of Cardiology, Japanese Heart Failure Society and Writing Committee of the Universal Definition of Heart Failure: Endorsed by the Canadian Heart Failure Society, Heart Failure Association of India, Cardiac Society of Australia and New Zealand, and Chinese Heart Failure Association. European journal of heart failure 2021;23:352-380.
7. Caraballo C, Desai NR, Mulder H et al. Clinical Implications of the New York Heart Association Classification. Journal of the American Heart Association 2019;8:e014240.
8. McMurray JJ, Packer M, Desai AS et al. Angiotensin-neprilysin inhibition versus enalapril in heart failure. The New England journal of medicine 2014;371:993-1004.
9. McMurray JJV, Solomon SD, Inzucchi SE et al. Dapagliflozin in Patients with Heart Failure and Reduced Ejection Fraction. The New England journal of medicine 2019;381:1995-2008.
10. Packer M, Anker SD, Butler J et al. Cardiovascular and Renal Outcomes with Empagliflozin in Heart Failure. The New England journal of medicine 2020;383:1413-1424.
11. Armstrong PW, Pieske B, Anstrom KJ et al. Vericiguat in Patients with Heart Failure and Reduced Ejection Fraction. The New England journal of medicine 2020;382:1883-1893.
12. Packer M. Heart Failure With a Mid-Range Ejection Fraction: A Disorder That a Psychiatrist Would Love. JACC Heart Fail 2017;5:805-807.
13. Butler J, Anker SD, Packer M. Redefining Heart Failure With a Reduced Ejection Fraction. Jama 2019;322:1761-1762.
14. Krayenbuhl HP, Bussmann WD, Turina M, Luthy E. Is the ejection fraction an index of myocardial contractility? Cardiologia 1968;53:1-10.
15. Abawi D, Rinaldi T, Faragli A et al. The non-invasive assessment of myocardial work by pressure-strain analysis: clinical applications. Heart Failure Reviews 2021.
16. Burkhoff D, Mirsky I, Suga H. Assessment of systolic and diastolic ventricular properties via pressure-volume analysis: a guide for clinical, translational, and basic researchers. American journal of physiology Heart and circulatory physiology 2005;289:H501-12.
17. Baan J, van der Velde ET, de Bruin HG et al. Continuous measurement of left ventricular volume in animals and humans by conductance catheter. Circulation 1984;70:812-23.
18. Suga H. Total mechanical energy of a ventricle model and cardiac oxygen consumption. The American journal of physiology 1979;236:H498-505.
19. Fincke R, Hochman JS, Lowe AM et al. Cardiac power is the strongest hemodynamic correlate of mortality in cardiogenic shock: a report from the SHOCK trial registry. Journal of the American College of Cardiology 2004;44:340-8.
20. Amzulescu MS, De Craene M, Langet H et al. Myocardial strain imaging: review of general principles, validation, and sources of discrepancies. European Heart Journal - Cardiovascular Imaging 2019;20:605-619.

21. Schuster A, Hor KN, Kowallick JT, Beerbaum P, Kutty S. Cardiovascular Magnetic Resonance Myocardial Feature Tracking: Concepts and Clinical Applications. Circ Cardiovasc Imaging 2016;9:e004077.
22. Pedrizzetti G, Claus P, Kilner PJ, Nagel E. Principles of cardiovascular magnetic resonance feature tracking and echocardiographic speckle tracking for informed clinical use. J Cardiovasc Magn Reson 2016;18:51.
23. Schneeweis C, Lapinskas T, Schnackenburg B et al. Comparison of myocardial tagging and feature tracking in patients with severe aortic stenosis. J Heart Valve Dis 2014;23:432-40.
24. Schneeweis C, Qiu J, Schnackenburg B et al. Value of additional strain analysis with feature tracking in dobutamine stress cardiovascular magnetic resonance for detecting coronary artery disease. Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance 2014;16:72.
25. Morton G, Schuster A, Jogiya R, Kutty S, Beerbaum P, Nagel E. Inter-study reproducibility of cardiovascular magnetic resonance myocardial feature tracking. J Cardiovasc Magn Reson 2012;14:43.
26. Lindsey ML, Kassiri Z, Virag JAI, de Castro Bras LE, Scherrer-Crosbie M. Guidelines for measuring cardiac physiology in mice. American journal of physiology Heart and circulatory physiology 2018;314:H733-H752.
27. Linscheid N, Santos A, Poulsen PC et al. Quantitative proteome comparison of human hearts with those of model organisms. PLoS Biol 2021;19:e3001144.
28. Wolfram J, Ferrari M. Clinical cancer nanomedicine. Nano Today 2019;25:85-98.
29. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nat Rev Mater 2021:1-17.
30. Iafisco M, Alogna A, Miragoli M, Catalucci D. Cardiovascular nanomedicine: the route ahead. Nanomedicine (Lond) 2019;14:2391-2394.
31. Morgan TT, Muddana HS, Altinoglu EI et al. Encapsulation of organic molecules in calcium phosphate nanocomposite particles for intracellular imaging and drug delivery. Nano Lett 2008;8:4108-15.
32. Kester M, Heakal Y, Fox T et al. Calcium phosphate nanocomposite particles for in vitro imaging and encapsulated chemotherapeutic drug delivery to cancer cells. Nano Lett 2008;8:4116-21.
33. Altinoglu EI, Russin TJ, Kaiser JM et al. Near-infrared emitting fluorophore-doped calcium phosphate nanoparticles for in vivo imaging of human breast cancer. ACS Nano 2008;2:2075-84.
34. Dorozhkin SV, Epple M. Biological and medical significance of calcium phosphates. Angew Chem Int Ed Engl 2002;41:3130-46.
35. Di Mauro V, Iafisco M, Salvarani N et al. Bioinspired negatively charged calcium phosphate nanocarriers for cardiac delivery of MicroRNAs. Nanomedicine 2016;11:891-906.
36. Rusconi F, Ceriotti P, Miragoli M et al. Peptidomimetic Targeting of Cavbeta2 Overcomes Dysregulation of the L-Type Calcium Channel Density and Recovers Cardiac Function. Circulation 2016;134:534-46.
37. Miragoli M, Ceriotti P, Iafisco M et al. Inhalation of peptide-loaded nanoparticles improves heart failure. Sci Transl Med 2018;10.
38. van Diepen S, Katz JN, Albert NM et al. Contemporary Management of Cardiogenic Shock: A Scientific Statement From the American Heart Association. Circulation 2017;136:e232-e268.
39. Kolte D, Khera S, Aronow WS et al. Trends in incidence, management, and outcomes of cardiogenic shock complicating ST-elevation myocardial infarction in the United States. Journal of the American Heart Association 2014;3:e000590.
40. Goldberg RJ, Makam RC, Yarzebski J, McManus DD, Lessard D, Gore JM. Decade-Long Trends (2001-2011) in the Incidence and Hospital Death Rates Associated with the In-Hospital Development of Cardiogenic Shock after Acute Myocardial Infarction. Circ Cardiovasc Qual Outcomes 2016;9:117-25.
41. Jeger RV, Radovanovic D, Hunziker PR et al. Ten-year trends in the incidence and treatment of cardiogenic shock. Ann Intern Med 2008;149:618-26.

42. Aissaoui N, Puymirat E, Tabone X et al. Improved outcome of cardiogenic shock at the acute stage of myocardial infarction: a report from the USIK 1995, USIC 2000, and FAST-MI French nationwide registries. European heart journal 2012;33:2535-43.
43. Burkhoff D, Naidu SS. The science behind percutaneous hemodynamic support: a review and comparison of support strategies. Catheter Cardiovasc Interv 2012;80:816-29.
44. Hajjar LA, Teboul JL. Mechanical Circulatory Support Devices for Cardiogenic Shock: State of the Art. Critical care 2019;23:76.
45. Burkhoff D, Sayer G, Doshi D, Uriel N. Hemodynamics of Mechanical Circulatory Support. Journal of the American College of Cardiology 2015;66:2663-2674.
46. Tschope C, Spillmann F, Potapov E et al. The "TIDE"-Algorithm for the Weaning of Patients With Cardiogenic Shock and Temporarily Mechanical Left Ventricular Support With Impella Devices. A Cardiovascular Physiology-Based Approach. Front Cardiovasc Med 2021;8:563484.
47. Ma P, Zhang Z, Song T et al. Combining ECMO with IABP for the treatment of critically Ill adult heart failure patients. Heart Lung Circ 2014;23:363-8.
48. Bastos MB, van Wiechen MP, Van Mieghem NM. PulseCath iVAC2L: next-generation pulsatile mechanical circulatory support. Future Cardiol 2020;16:103-112.
49. Tschope C, Van Linthout S, Klein O et al. Mechanical Unloading by Fulminant Myocarditis: LV-IMPELLA, ECMELLA, BI-PELLA, and PROPELLA Concepts. J Cardiovasc Transl Res 2019;12:116-123.
50. Kapur NK, Davila CD, Chweich H. Protecting the Vulnerable Left Ventricle: The Art of Unloading With VA-ECMO. Circulation Heart failure 2019;12:e006581.
51. Spillmann F, Van Linthout S, Schmidt G et al. Mode-of-action of the PROPELLA concept in fulminant myocarditis. European heart journal 2019;40:2164-2169.
52. Knierim J, Heck R, Pieri M et al. Outcomes from a recovery protocol for patients with continuous-flow left ventricular assist devices. J Heart Lung Transplant 2019;38:440-448.
53. Fiser SM, Tribble CG, Kaza AK et al. When to discontinue extracorporeal membrane oxygenation for postcardiotomy support. Ann Thorac Surg 2001;71:210-4.
54. Ortuno S, Delmas C, Diehl JL et al. Weaning from veno-arterial extra-corporeal membrane oxygenation: which strategy to use? Ann Cardiothorac Surg 2019;8:E1-E8.
55. Feldman D, Pamboukian SV, Teuteberg JJ et al. The 2013 International Society for Heart and Lung Transplantation Guidelines for mechanical circulatory support: executive summary. J Heart Lung Transplant 2013;32:157-87.
56. Thiele H, Freund A, Gimenez MR et al. Extracorporeal life support in patients with acute myocardial infarction complicated by cardiogenic shock - Design and rationale of the ECLS-SHOCK trial. American heart journal 2021;234:1-11.
57. Abawi D, Faragli A, Schwarzl M et al. Cardiac power output accurately reflects external cardiac work over a wide range of inotropic states in pigs. BMC cardiovascular disorders 2019;19:217.
58. Faragli A, Tanacli R, Kolp C et al. Cardiovascular magnetic resonance feature tracking in pigs: a reproducibility and sample size calculation study. The International Journal of Cardiovascular Imaging 2020;36:703-712.
59. Faragli A, Tanacli R, Kolp C et al. Cardiovascular magnetic resonance-derived left ventricular mechanics-strain, cardiac power and end-systolic elastance under various inotropic states in swine. J Cardiovasc Magn Reson 2020;22:79.
60. Cotter G, Williams SG, Vered Z, Tan LB. Role of cardiac power in heart failure. Current opinion in cardiology 2003;18:215-22.
61. Milnor WR, Conti CR, Lewis KB, O'Rourke MF. Pulmonary arterial pulse wave velocity and impedance in man. Circulation research 1969;25:637-49.
62. Borlaug BA, Kass DA. Ventricular-vascular interaction in heart failure. Heart failure clinics 2008;4:23-36.
63. Laskey WK, Ferrari VA, Palevsky HI, Kussmaul WG. Ejection characteristics in primary pulmonary hypertension. Am J Cardiol 1993;71:1111-4.
64. Shah MR, Hasselblad V, Stevenson LW et al. Impact of the pulmonary artery catheter in critically ill patients: meta-analysis of randomized clinical trials. Jama 2005;294:1664-70.

65. Katz LN, Feinberg H. The relation of cardiac effort to myocardial oxygen consumption and coronary flow. Circulation research 1958;6:656-69.
66. Gobel FL, Norstrom LA, Nelson RR, Jorgensen CR, Wang Y. The rate-pressure product as an index of myocardial oxygen consumption during exercise in patients with angina pectoris. Circulation 1978;57:549-56.
67. Russell K, Eriksen M, Aaberge L et al. A novel clinical method for quantification of regional left ventricular pressure-strain loop area: a non-invasive index of myocardial work. European heart journal 2012;33:724-33.
68. Barbier P, Mirea O, Cefalu C, Maltagliati A, Savioli G, Guglielmo M. Reliability and feasibility of longitudinal AFI global and segmental strain compared with 2D left ventricular volumes and ejection fraction: intra- and inter-operator, test-retest, and inter-cycle reproducibility. Eur Heart J Cardiovasc Imaging 2015;16:642-52.
69. Abou R, van der Bijl P, Bax JJ, Delgado V. Global longitudinal strain: clinical use and prognostic implications in contemporary practice. Heart 2020;106:1438-1444.
70. Marwick TH, Shah SJ, Thomas JD. Myocardial Strain in the Assessment of Patients With Heart Failure: A Review. JAMA Cardiol 2019;4:287-294.
71. Claus P, Omar AMS, Pedrizzetti G, Sengupta PP, Nagel E. Tissue Tracking Technology for Assessing Cardiac Mechanics: Principles, Normal Values, and Clinical Applications. JACC Cardiovascular imaging 2015;8:1444-1460.
72. Schuster A, Paul M, Bettencourt N et al. Myocardial feature tracking reduces observer-dependence in low-dose dobutamine stress cardiovascular magnetic resonance. PloS one 2015;10:e0122858.
73. Schuster A, Morton G, Hussain ST et al. The intra-observer reproducibility of cardiovascular magnetic resonance myocardial feature tracking strain assessment is independent of field strength. European journal of radiology 2013;82:296-301.
74. Lapinskas T, Grune J, Zamani SM et al. Cardiovascular magnetic resonance feature tracking in small animals - a preliminary study on reproducibility and sample size calculation. BMC medical imaging 2017;17:51.
75. Lindsey ML, Kassiri Z, Virag JAI, Brás LEdC, Scherrer-Crosbie M. Guidelines for measuring cardiac physiology in mice. American Journal of Physiology-Heart and Circulatory Physiology 2018;314:H733-H752.
76. Sampath S, Parimal AS, Feng D et al. Quantitative MRI biomarkers to characterize regional left ventricular perfusion and function in nonhuman primates during dobutamine-induced stress: A reproducibility and reliability study. Journal of magnetic resonance imaging : JMRI 2017;45:556-569.
77. Onishi T, Saha SK, Delgado-Montero A et al. Global longitudinal strain and global circumferential strain by speckle-tracking echocardiography and feature-tracking cardiac magnetic resonance imaging: comparison with left ventricular ejection fraction. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography 2015;28:587-96.
78. Eitel I, Stiermaier T, Lange T et al. Cardiac Magnetic Resonance Myocardial Feature Tracking for Optimized Prediction of Cardiovascular Events Following Myocardial Infarction. JACC Cardiovascular imaging 2018;11:1433-1444.
79. Mangion K, Carrick D, Carberry J et al. Circumferential Strain Predicts Major Adverse Cardiovascular Events Following an Acute ST-Segment-Elevation Myocardial Infarction. Radiology 2018:181253.
80. Romano S, Judd RM, Kim RJ et al. Feature-Tracking Global Longitudinal Strain Predicts Death in a Multicenter Population of Patients With Ischemic and Nonischemic Dilated Cardiomyopathy Incremental to Ejection Fraction and Late Gadolinium Enhancement. JACC Cardiovascular imaging 2018;11:1419-1429.
81. Reichek N, Wilson J, St John Sutton M, Plappert TA, Goldberg S, Hirshfeld JW. Noninvasive determination of left ventricular end-systolic stress: validation of the method and initial application. Circulation 1982;65:99-108.

82. Rhea IB, Rehman S, Jarori U, Choudhry MW, Feigenbaum H, Sawada SG. Prognostic utility of blood pressure-adjusted global and basal systolic longitudinal strain. Echo research and practice 2016;3:17-24.
83. Yingchoncharoen T, Agarwal S, Popovic ZB, Marwick TH. Normal ranges of left ventricular strain: a meta-analysis. J Am Soc Echocardiogr 2013;26:185-91.
84. Weiner RB, Weyman AE, Kim JH, Wang TJ, Picard MH, Baggish AL. The impact of isometric handgrip testing on left ventricular twist mechanics. J Physiol 2012;590:5141-5150.
85. Stokke TM, Hasselberg NE, Smedsrud MK et al. Geometry as a Confounder When Assessing Ventricular Systolic Function: Comparison Between Ejection Fraction and Strain. Journal of the American College of Cardiology 2017;70:942-954.
86. Rajagopalan S, Al-Kindi SG, Brook RD. Air Pollution and Cardiovascular Disease: JACC State-of-the-Art Review. Journal of the American College of Cardiology 2018;72:2054-2070.
87. Patton JS, Fishburn CS, Weers JG. The lungs as a portal of entry for systemic drug delivery. Proc Am Thorac Soc 2004;1:338-44.
88. Irngartinger M, Camuglia V, Damm M, Goede J, Frijlink HW. Pulmonary delivery of therapeutic peptides via dry powder inhalation: effects of micronisation and manufacturing. European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV 2004;58:7-14.
89. Elhissi A. Liposomes for Pulmonary Drug Delivery: The Role of Formulation and Inhalation Device Design. Current pharmaceutical design 2017;23:362-372.
90. Tsyganenko D, Gromann TW, Schoenrath F et al. Predictors of mid-term outcomes in patients undergoing implantation of a ventricular assist device directly after extracorporeal life support. Eur J Cardiothorac Surg 2019;55:773-779.
91. Dangers L, Brechot N, Schmidt M et al. Extracorporeal Membrane Oxygenation for Acute Decompensated Heart Failure. Critical care medicine 2017;45:1359-1366.
92. Aissaoui N, El-Banayosy A, Combes A. How to wean a patient from veno-arterial extracorporeal membrane oxygenation. Intensive Care Med 2015;41:902-5.
93. Aissaoui N, Luyt CE, Leprince P et al. Predictors of successful extracorporeal membrane oxygenation (ECMO) weaning after assistance for refractory cardiogenic shock. Intensive Care Med 2011;37:1738-45.
94. Pappalardo F, Pieri M, Arnaez Corada B et al. Timing and Strategy for Weaning From Venoarterial ECMO are Complex Issues. J Cardiothorac Vasc Anesth 2015;29:906-11.
95. Smith M, Vukomanovic A, Brodie D, Thiagarajan R, Rycus P, Buscher H. Duration of veno-arterial extracorporeal life support (VA ECMO) and outcome: an analysis of the Extracorporeal Life Support Organization (ELSO) registry. Critical care 2017;21:45.
96. Durinka JB, Bogar LJ, Hirose H et al. End-organ recovery is key to success for extracorporeal membrane oxygenation as a bridge to implantable left ventricular assist device. ASAIO J 2014;60:189-92.
97. Cavarocchi NC, Pitcher HT, Yang Q et al. Weaning of extracorporeal membrane oxygenation using continuous hemodynamic transesophageal echocardiography. J Thorac Cardiovasc Surg 2013;146:1474-9.

Eidesstattliche Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,
- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

…18.07.2022……..

Datum Unterschrif