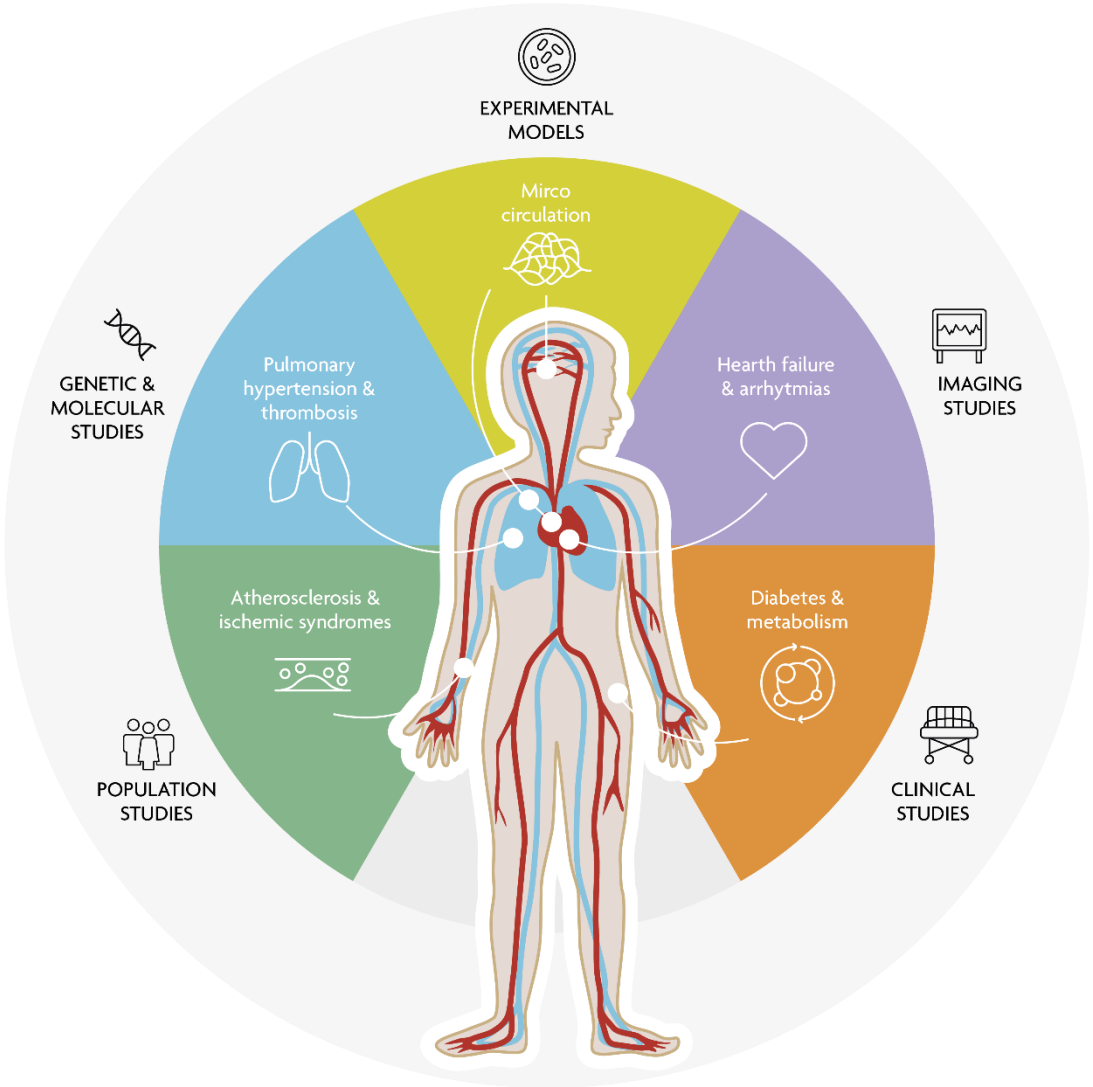
Amsterdam UMC
Universitair Medische Centra

Amsterdam Cardiovascular Sciences

9th Annual ACS symposium

Thursday December 7, 2023

Felix Meritis, Keizersgracht 324, Amsterdam



Content

General information

Meeting venue

Felix Meritis

Keizersgracht 324

1016 EZ Amsterdam

Homepage - Cultuurhuis Felix Meritis Amsterdam

Meeting rooms

Plenary sessions will take place at Concertzaal (ground floor)

Lunch, break and reception will take place at Zuilenzaal (second floor)

Poster sessions will take place at Shaffyzaal (fifth floor)

Internet access

WiFi is freely available

Contact

Organizing committee: acs@amsterdamumc.nl

Keynote speakers

Prof. dr. Norbert Hübner

Max Delbrück Center, Berlin, Germany

Keynote lecture 1: 09.05-09.50

Novel, Short, Small & Young: microproteins in the human heart and beyond

Dr. Norbert Hübner is professor and chair in Cardiovascular Sciences at Charité Medical School and senior group head at the Max-Delbrueck-Center (MDC) for Molecular Medicine in Berlin, Germany. His research is focused on studying cardiovascular biology in health and disease. His general approach is to dissect genetic networks across the scale from molecular to physiological in human cardiac tissues and cells.

Dr. Hübner has extensive experience in chairing scientific programs, working with scientific bodies, and have led several multi-institutional research consortia. He is currently Speaker of the Max-Delbruck-Center and Helmholtz Society Program on Systems Medicine and Cardiovascular Disease with approx. 90 junior and senior research groups. Over the past 10 years he guided two large EU funded consortia as a member of the Steering Committee (EURAtools) and as coordinator (EURATRANS).

Currently, dr. Hübner is the European coordinator of a Fondation Leducq Transatlantic Network of Excellence to identify the cellular and molecular drivers of cardiac fibrosis and a recipient of an ERC advanced grant.

Prof. dr. Peter Libby

Brigham and Women's Hospital, Harvard Medical School, Boston, USA

Keynote lecture 2: 11.25-12.10

Clonal Hematopoiesis and Cardiovascular Disease

Dr. Peter Libby is a cardiovascular medicine specialist at Brigham and Women's Hospital (BWH) and the Mallinckrodt Professor of Medicine at Harvard Medical School (HMS). He completed a residency in internal medicine and a fellowship in cardiovascular disease at Peter Bent Brigham Hospital (now BWH). He also completed a research fellowship in cellular physiology at HMS. Dr. Libby is board certified in internal medicine and cardiovascular disease.

The author of some 400 original peer-reviewed publications, some 450 reviews, chapters, or other publications, Dr. Libby also serves as an Editor of the leading textbook of cardiovascular medicine. Dr. Libby's clinical and research interests include vascular biology, atherosclerosis and preventive cardiology. The research laboratory that Dr. Libby directs studies the messengers created by the body that may produce arterial plaque and blockages, as well as normal and abnormal function of smooth muscle and endothelial cells. Dr. Libby is perennially named a top cardiologist. His research has received funding from the American Heart Association and National Institutes of Health. Dr. Libby has received research recognitions on four continents including the highest research awards from the American Heart Association and American College of Cardiology, the Gold Medal of the European Society of Cardiology, the Anitschkow award from the European Atherosclerosis Society, The Ernst Jung Gold Medal for Medicine, and the Earl Benditt award for vascular biology.

Dr. Richard van Duin

Dutch Heart Foundation

Keynote lecture 3: 16.25-17.00

Science communication: Breaking the black box

As a science communication advisor for the Dutch Heart Foundation he wrote dozens of articles for newspapers, websites, magazines and social media. His mission? To inspire, educate and entertain people with compelling, clear stories about science. Because science should be for everybody to enjoy.

Program

08.15-09.00	Registration
09.00-09.50	**Opening** Chairs: dr. Malou van den Boogaard & dr. Vincent Jongkind Concertzaal
09.00-09.05	Introduction by ACS directors: prof. dr. Jolanda van der Velden & prof. dr. Arthur Wilde
09.05-09.50	**Keynote 1: Prof. dr. Norbert Hübner (Max Delbrück Center Berlin)** *Novel, Short, Small & Young: microproteins in the human heart and beyond*
09.50-10.15	**Orals session 1** Chairs: dr. Nick van Es & Stefan Smorenburg Concertzaal
	1 Erik Duijvelaar *Empagliflozin improves mitochondrial biogenesis and ameliorates vascular remodeling in experimental pulmonary arterial hypertension*
	2 Eva Aalbregt *Four Dimensional Flow MRI-derived Hemodynamics in Abdominal Aortic Aneurysms: Reproducibility and Associations with Diameter, Intraluminal Thrombus Volume and Vorticity*
10.15-10.30	**Pitch poster session 1 (Even abstract numbers)** Chair: dr. Annette Neele Concertzaal
	10 Alexandra Giovou *Postnatal ventricular regeneration in congenital heart disease: function and application of TBX5 for promoting cardiomyocyte proliferation*
	12 Thomas Steunenberg *Clinical outcomes of 5 000 IU heparin versus activated clotting time guided heparinization during non-cardiac arterial procedures: a propensity score matched analysis*
	14 Beau Neep *Understanding the role of SOX17 in the shear stress response of the arterial endothelium and its implication in early Pulmonary Arterial Hypertension development*
	16 Benthe Ariëns *The effect of SGLT2-inhibition on the kidney perfusion and diffusion in patients with T2D*
10.30-11.25	**Poster session1 with coffee (Even abstract numbers)** Shaffyzaal

11.25-12.10	**Keynote 2: Prof. dr. Peter Libby, MD (Brigham and Women's Hospital and Harvard Medical School, United States)** *Clonal Hematopoiesis and Cardiovascular Disease* Chairs: prof. dr. Fabrice Martens & dr. Jeffrey Kroon Concertzaal
12.10-12.30	**Pitch poster session 2 (Odd abstract numbers)** Chair: dr. Vincent Jongkind Concertzaal
	9 Mick Renkens *The Enduring Hiatus Between Clinical Guidelines and Clinical Practice in Appropriate Lipid-Lowering Therapy Still Affects a Third of Patients Undergoing PCI in Europe: Insights from PIONEER IV and Multivessel Talent Trials*
	11 Anoek Rooijakkers *Long-range gene editing of LMNA*
	13 Carolien Volleman *Endothelial activation in COVID-19 induced ARDS patients on ECMO support: a pilot study*
	15 Sarah Hilderink *Hypertrophic cardiomyopathy evident at an early age in knock-in mice with the most common Dutch founder mutation, MYBPC3c.2373InsG*
	17 Jordan Kraaijenhof *Targeting apolipoprotein C-III with olezarsen leads to significant reduction in fasted and postprandial triglyceride levels*
	19 Rushd Al-Shama *Myeloperoxidase causes arrhythmogenic remodeling in cardiac slices*
12.30-13.30	**Lunch** Zuilenzaal
13.30-14.15	**Poster session 2 (odd abstract numbers)** Shaffyzaal
14.15-14.45	**Plenary session: Valorisation** Chair: prof. dr. Folkert Asselbergs Concertzaal
14.15-14.30	**Philip Croon** *DGLT Health: utilizing artificial intelligence to accommodate 20,000 holters per year*
14.30-14.45	**Prof. dr. Dave Koolbergen** *The Haermonics story*
14.45-15.30	**Orals session 2** Chairs: dr. Marielle van de Veerdonk & dr. Elena Rampanelli Concertzaal
	3 Lieve van der Maarel *Altered chromatin conformation drives ectopic PITX2 expression and sinoatrial node dysfunction in unrelated families presenting with a novel cardiac syndrome*
	4 Luca Deursen *Microvascular endothelial dysfunction in skin is associated with higher risk of heart failure with preserved ejection fraction in women with type 2 diabetes: the Hoorn Diabetes Care System Cohort*

	6 Ed Eringa *Peritoneal dialysis aggravates and accelerates atherosclerosis in uraemic ApoE-/- mice*
15.30-16.00	Break Zuilenzaal
16.00 - 16.25	**Orals session 3** Chairs: dr. Nick van Es & Stefan Smorenburg Concertzaal
	7 Sha Chen *Empagliflozin prevents diastolic and systolic dysfunction in heart failure and reduces cardiac NHE1 activity, independent of SGLT2*
	8 Mitchel Molenaar *Machine learning for risk stratification using clinical and echocardiography data in patients with chronic coronary syndrome*
16.25-17.00	**Closing session** Chairs: dr. Annette Neele & dr. Malou van den Boogaard Concertzaal
16.25-16.55	**Dr. Richard van Duin (Dutch Heart Foundation)** *Science communication: Breaking the black box*
16.55-17.00	Introduction new ACS directors dr. Ronak Delewi & prof. dr. Menno de Winther
17.00-17.05	Awards ceremony
17.05-18.00	**Reception** Zuilenzaal

Oral Abstract 1

Presenter: Erik Duijvelaar

Pulmonary Hypertension & Thrombosis

Empagliflozin improves mitochondrial biogenesis and ameliorates vascular remodeling in experimental pulmonary arterial hypertension

E. Duijvelaar, K. Yoshida, E. N. Toth, X. Q. Sun, B. Neep, X. Pan, T. Jujo, Y. Yoshida, J. Aman, F. S. Handoko-De Man, H. J. Bogaard

Pulmonary Medicine Location VUmc

Introduction: Pulmonary arterial hypertension (PAH) is a devastating disease in which narrowing of the pulmonary vasculature often leads to right ventricular (RV) failure. Empagliflozin (EMPA), a sodium-glucosecotransporter 2 (SGLT2) inhibitor, provides well-established clinical benefits for patients with heart failure.

Aims and Objective: To investigate the effects of EMPA in vitro and in vivo.

Methods: Sprague-Dawley rats were injected with 25 mg/kg Sugen 5416 followed by 3 weeks of 10% oxygen exposure (SuHx) to induce PAH, and subsequently randomised to 300 mg/kg EMPA (n=12) or placebo (n=12). After 4 weeks of treatment, echocardiography and right heart catheterization (RHC) were performed. The rats were sacrificed for histological evaluation. Pulmonary microvascular endothelial cells (MVEC) from PAH patients and controls, were treated with 1 µM EMPA.

Results: In a SuHx PAH rat model, EMPA attenuated pulmonary vascular resistance (PVR) and pulmonary arterial pressure. EMPA treatment reversed RV dilatation and wall thickening. EMPA reduced RV fibrosis and attenuated vascular occlusion, in particular by reducing intimal thickness. Heart rate and systemic blood pressures were unaffected. In MVECs of PAH patients, EMPA treatment, and SGLT2 knockdown, increased the expression of peroxisome proliferator-activated receptor gammacoactivator-1α (PGC-1α), a master regulator of mitochondrial biogenesis. In addition, EMPA attenuated mitochondrial oxidative stress and MVEC proliferation.

Conclusion: Empagliflozin ameliorated pulmonary vascular remodeling and improved right ventricular hemodynamics in a rat model of PAH. These effects could be mediated by improvement in mitochondrial biogenesis and attenuation of hyperproliferation.

Oral Abstract 2

Presenter: Eva Aalbregt

Microcirculation

Four Dimensional Flow MRI-derived Hemodynamics in Abdominal Aortic Aneurysms: Reproducibility and Associations with Diameter, Intraluminal Thrombus Volume and Vorticity

Eva Aalbregt, Reza Indrakusuma, Hamid Jalalzadeh, R. Nils Planken, Ron Balm, Aart J. Nederveen, Kak Khee Yeung, Pim van Ooij

Vascular Surgery | Radiology and Nuclear Medicine Location AMC

Background: Maximum diameter measurements are currently used to assess the rupture risk of abdominal aortic aneurysms (AAAs); however, these are not precise enough to prevent rupture. 4D flow magnetic resonance imaging (MRI) derived parameters provide additional information to assess hemodynamics of AAAs. The aim of this research was to assess the reproducibility of 4D flow MRI-derived hemodynamics and investigate possible correlations with morphological parameters.

Methods: A total of 19 (18 male) asymptomatic AAA patients with a maximum diameter > 30 mm and mean age 72.3 ± 5.9 years were scanned twice with a one week interval with a 3D free-breathing 4D flow MRI phase-contrast acquisition with retrospective ECG-gating at 3.0T. Aortic volumes were segmented at peak systole. Reproducibility was assessed by voxel-by-voxel analysis after registration. Mean flow velocity, mean wall shear stress (WSS) and mean lumen diameter were assessed. In addition, the maximum diameter, intraluminal thrombus (ILT) volume, qualitative vorticity scores and growth rate were studied using additional data. For reproducibility assessment, Bland-Altman analyses and orthogonal regression were conducted. Spearman's correlation coefficients were calculated for non-normal distributed variables. Potential correlations between hemodynamics and vorticity scores were assessed using linear regression with dummy variables. Statistical significance was set at a two-tailed p-value of <0.05.

Results: Within the reproducibility analyses the Pearson correlation coefficients for respectively flow velocity and WSS were 0.82 ± 0.08 m/s and 0.81 ± 0.09 Pa. Mean WSS correlated with mean flow velocity ($R=0.77$, $p<0.001$) and inversely correlated with mean lumen diameter ($R=-0.65$, $p<0.01$). No significant associations were found between 4D flow-derived hemodynamic parameters and maximum diameter, ILT volume, vorticity scores or growth rate.

Conclusion: 4D flow MRI is robust for assessing the hemodynamics within AAAs. No correlations were found between 4D flow MRI-derived hemodynamic and morphologic parameters, vorticity scores and growth rate.

Oral Abstract 3

Presenter: Lieve van der Maarel

Heart Failure & Arrhythmias

Altered chromatin conformation drives ectopic PITX2 expression and sinoatrial node dysfunction in unrelated families presenting with a novel cardiac syndrome

Lieve E. van der Maarel, Fernanda Bosada, Ridwane Mungroo, Frederik H. T. Tiel Groenestege, Harsha D. Devalla, Vincent M. Christoffels

Medical Biology Location AMC

Several families sharing overlapping deletions in a 1.5 Megabase pair gene desert on chromosome 4q25 present with a complex, heterogeneous cardiac syndrome including sinoatrial node (SAN) dysfunction, atrial fibrillation and atrial septum defects. These deletions remove evolutionarily conserved CTCF binding sites that physically separate a sub-topologically associating domain (TAD) containing the transcription factor PITX2 from a sub-TAD harboring noncoding RNA genes. Hi-C analysis in hiPSC-derived cardiomyocytes revealed that the deletion of this region fuses these sub-TADs, rewiring local DNA interactions.

We generated mice lacking the genomic region orthologous to the smallest genomic region deleted in patients. These mice recapitulated the SAN dysfunction and atrial arrhythmogenesis observed in patients. Expression analysis of left and right atrial tissue revealed that of the genes in the genomic regions flanking the deletion, only Pitx2 was deregulated in the heart. Normally, Pitx2 expression is restricted to the left side of the developing heart, driving left atrial and sinus venosus morphogenesis and preventing the formation of a left-sided SAN. We found that Pitx2c was selectively ectopically expressed in pacemaker cardiomyocytes of the developing and postnatal SAN. Tbx3, Isl1 and Shox2, transcription factors that drive pacemaker cell differentiation, were gradually downregulated during SAN development in mutant mice. The gradual loss of the pacemaker gene program coincided with the activation of the atrial myocardial gene program in approximately half of the pacemaker cardiomyocytes. RNA sequencing of SAN tissue revealed reduced expression of many pacemaker cardiomyocyte-specific genes and gained expression of working myocardium-associated genes.

We conclude that the deletion of CTCF binding sites rewires a long-range regulatory architecture that deregulates PITX2 expression in the SAN, inducing a phenotypic transformation towards an atrial myocardial phenotype in a subset of pacemaker cells and driving SAN dysfunction in affected patients.

Oral Abstract 4

Presenter: Luca van Deursen

Diabetes & Metabolism; Microcirculation; Heart Failure & Arrhythmias

Microvascular endothelial dysfunction in skin is associated with higher risk of heart failure with preserved ejection fraction in women with type 2 diabetes: the Hoorn Diabetes Care System Cohort

Elisa Dal Canto† , L. van Deursen†, A. G. Hoek, P. J. M. Elders, H. M. den Ruijter, J. van der Velden, V. van Empel, E. H. Serné, E. C. Eringa and J. W.J. Beulens**

Physiology Location Vumc

Background: Microvascular dysfunction plays a crucial role in complications of type 2 diabetes and might contribute to heart failure with preserved ejection fraction (HFpEF), a disease that disproportionally affects women.

Aim: We aimed to investigate if presence and degree of microvascular dysfunction (MVD) in skin relates to markers of left ventricular diastolic dysfunction (LVDD) and HFpEF risk in adults with type 2 diabetes, and whether sex modifies this association.

Methods: We recruited 154 participants (50% women) from the Hoorn Diabetes Care System Cohort, a prospective cohort study, for in vivo evaluation of skin MVD, echocardiography and blood sampling. MVD was assessed by laser speckle contrast analysis combined with iontophoresis of insulin, acetylcholine and sodium nitroprusside (SNP). We performed a cross-sectional analysis of the association between perfusion responses and echocardiographic and clinical markers of LVDD and the H2FPEF score by multivariable linear regression analysis adjusted for confounders. Sex was evaluated as a potential effect modifier and the analysis was stratified.

Results: Mean age was 67±6y, mean HbA1c 7.6±1.3%. Women were more frequently obese (54.5 vs. 35.1%), had higher NT-proBNP plasma levels (80, IQR:34–165 vs. 46, 27–117 pg/ml) and E/E'(13.3 ± 4.3 vs. 11.4 ± 3.0) than men. Eleven women and three men were diagnosed with HFpEF, and showed lower perfusion response to insulin than those without HFpEF. A lower perfusion response to insulin and acetylcholine was associated with higher HFpEF risk in women, but not men (10% decreased perfusion response was associated with 5.8% [95%CI: 2.3;9.4%] and 5.9% [1.7;10.1%] increase of the H2FPEF score, respectively). A lower perfusion response to SNP was associated with higher pulmonary arterial systolic pressure in men while a lower perfusion response to acetylcholine associated with higher LV mass index in women and with worse LV longitudinal strain in the total population. No significant associations were found between perfusion responses and conventional LVDD markers.

Conclusions: Impaired microvascular responses to insulin and acetylcholine in skin confers a higher risk of HFpEF in women with type 2 diabetes. In vivo measures of systemic MVD could represent novel risk markers for HFpEF, opening new avenues for the prevention of HFpEF in type 2 diabetes.

Oral Abstract 5

Presenter: Charlotte Teunis

Diabetes & Metabolism

Tryptophan Metabolites and Incident Cardiovascular Disease the EPIC-Norfolk Prospective Population Study

Charlotte J. Teunis, Erik S.G. Stroes, S. Matthijs Boekholdt, Nicholas J. Wareham, Andrew J. Murphy, Max Nieuwdorp, Stanley L. Hazen, Nordin M.J. Hanssen

Vascular Medicine Location AMC

Background: Cardiovascular disease (CVD) remains the largest cause of death globally due to various risk factors. One novel potential contributor to CVD might be the metabolism of the essential amino acid tryptophan (Trp), which through many pathways can produce immunomodulatory metabolites such as kynurenine, indole-3-propionate and serotonin.

Methods: We used the community-based EPIC-Norfolk cohort (46.3% men, age 59.8 ± 9.0) with a median follow-up of 22.3 (0-25.00) years to study associations between the relative levels of Trp metabolites measured with untargeted metabolomics and incident development of CVD. Serum from n=11972 apparently healthy subjects were analysed, of which 6982 individuals had developed CVD at the end of follow-up. Cox proportional hazard models were used to study associations, adjusted for sex, age, conventional cardiovascular risk factors and CRP. All metabolites were Ln-normalised prior to analysis.

Results: Higher levels of Trp were inversely associated with mortality (HR 0.73; CI 0.64-0.83) and fatal CVD (HR 0.76; CI 0.59-0.99). Higher levels of kynurenine (HR 1.33; CI 1.19-1.49) and the [Kynurenine]/[Tryptophan]-ratio (HR 1.24; CI 1.14-1.35) were associated with a higher incident development of CVD. Serotonin was not associated with overall CVD, but we did find associations for myocardial infarction and stroke.

Conclusion: Tryptophan levels were inversely correlated with CVD, while several of its major metabolites (especially kynurenine and serotonin) were positively correlated. These findings indicate that mechanistic studies are required to understand the role of Trp metabolism in CVD with the goal to identify new therapeutic targets.

Translational Perspective: Understanding the pathogenesis of CVD is urgently needed to cope with its rising incidence. We found that higher Trp concentrations are associated with a lower incidence of fatal CVD, while some downstream metabolites are associated with an increased incidence of fatal and non-fatal CVD. Concentrations of Trp-metabolites may be of interest as a diagnostic marker and modulating Trp-pathways may be a novel therapeutic target.

Oral Abstract 6

Presenter: Ed Eringa

Atherosclerosis & Ischemic Syndromes

Peritoneal dialysis aggravates and accelerates atherosclerosis in uraemic ApoE-/- mice

Jamie Kane, Winnie G Vos, Laura A Bosmans, Bram W van Os, Myrthe den Toom, Sanne Hoeksema-Hackmann, Denise Moen-de Wit, Marion J Gijbels, Linda Beckers, Aldo Grefhorst, Johannes H M Levels, Lily Jakulj, Marc G Vervloet, Esther Lutgens, Etto C Eringa

Physiology Location AMC

Background and Aims: Atherosclerosis is highly prevalent in people with chronic kidney disease (CKD) and including those receiving peritoneal dialysis (PD). Whilst being life-saving, PD induces profound systemic inflammation which may aggravate atherosclerosis. Therefore, the hypothesis is that PD aggravates atherosclerosis via immune cell activation.

Methods: ApoE-/- mice were subjected to a 5/6 nephrectomy to induce CKD (CKD). Three weeks later, mice were fed a high-cholesterol diet and nephrectomised mice received daily peritoneal infusions of 3.86% Physioneal® for 67 further days (CKD+PD) until the end of the experiment.

Results: CKD+PD mice displayed more severe atherosclerotic disease than control mice. Plaque area increased, and plaques were more advanced with a vulnerable phenotype typified by decreased collagen content and fibrous cap thickness. Increased CD3+ T-cell numbers were present in plaques and perivascular adipose tissue (PVAT) of CKD and CKD+PD mice. Plaques of CKD+PD mice contained more iNOS+ immune cells.

Spleens of CKD+PD mice showed more CD4+ central memory, terminally differentiated type 1 T-helper (Th1), Th17, and CX3C motif chemokine receptor 1+ (CX3CR1) CD4+ T-cells with less regulatory and effector T-cells.

Conclusions: PD-fluid exposure in uraemic mice potentiates systemic and vascular T-cell driven inflammation and aggravates atherosclerosis. PD caused polarisation of CD4+ T-cells towards an inflammatory Th1/Th17 phenotype, and increased CX3CR1+ CD4+ T-cells, which are associated to vascular homing in CKD-associated atherosclerosis. Targeting CD4+ T-cell activation and polarisation toward CX3CR1+ has the potential to treat atherosclerosis in CKD patients receiving PD.

Oral Abstract 7

Presenter: Sha Chen

Heart Failure & Arrhythmias

Empagliflozin prevents diastolic and systolic dysfunction in heart failure and reduces cardiac NHE1 activity, independent of SGLT2

Sha Chen, Qian Wang, Diane Bakker, Liping Zhang, Inge van der Made, Csenger Kovacshazi, Giricz Zoltan, Gabor Brenner, Mathilde Rivaud, Markus Hollmann, Nina Weber, Esther Creemers, Ruben Coronel, Coert J Zuurbier

Anesthesiology Location AMC

Background: SGLT2 inhibitors (SGLT2i) are now increasingly used in the treatment of heart failure (HF), although underlying mechanisms remain unknown. Inhibition of SGLT2 and inhibition of the sodium/hydrogen exchanger (NHE1) are two suggested mechanisms.

Research Question: Are the protective effects of SGLT2i in HF mediated through inhibition of the SGLT2 and/or the NHE1?

Methods: We created a SGLT2 KO mouse (C57/Bl6N) through CRISP/Cas. HF was induced in male and female WT and KO animals through transverse aortic constriction (TAC) and chronic subcutaneous deoxycorticosterone acetate (DOCA) after implantation of a pellet. Sham animals were also examined. Two days after surgery, animals received control (CO)- or empagliflozin (EMPA)-enriched chow. Echocardiography (E/A, E'/e, EF) was performed at baseline and 10 days after TAC/DOCA. Cardiac cells were isolated for determination of NHE1 activity employing the NH4+ pulse and pH monitoring with SNARF fluorescence.

Results: SGLT2 KO was confirmed at the DNA, mRNA and protein level, and functionally confirmed by glucosuria. Baseline cardiac function was similar between genotypes. TAC/DOCA induced similar diastolic dysfunction in both HF-WT and HF-KO animals. EMPA treatment prevented diastolic dysfunction in WT and KO animals. In addition, TAC/DOCA also induced mild systolic dysfunction, which was less for HF-KO vs HF-WT animals but which was equally prevented by EMPA. Similar protective effects of EMPA in WT and KO were observed for left ventricular or left atrial hypertrophy and lung and liver wet/dry ratio, whereas groups were not different for plasma glucose or ketones. NHE1 activity was increased in WT and KO HF groups, but normalized to baseline values in the EMPA-group .

Conclusions: We show for the first time that the cardioprotective effects of the SGLT2i EMPA in HF 1) on diastolic and systolic dysfunction are independent of SGLT2, and 2) are associated with the prevention of activated cardiac NHE1.

Oral Abstract 8

Presenter: Mitchel Molenaar

Atherosclerosis & Ischemic Syndromes

Machine learning for risk stratification using clinical and echocardiography data in patients with chronic coronary syndrome

Mitchel A. Molenaar; Berto J. Bouma; Folkert W. Asselbergs; Niels J. Verouden; Jasper L. Selder; Steven A.J. Chamuleau; Mark J. Schuuring

Cardiology Location AMC

Introduction: Risk stratification in patients with chronic coronary syndrome (CCS) primarily relies on echocardiographic evaluation of left ventricular (LV) function. Machine learning (ML) methods enables analysis of complex datasets including transthoracic echocardiography (TTE) studies. We aimed to evaluate the accuracy of ML using clinical and TTE data to predict all-cause five-year mortality in patients with CCS.

Methods: Data of consecutive patients with CCS were retrospectively collected if they attended the outpatient clinic of a tertiary center between 2015 and 2017 and underwent TTE assessment. A ML model (eXtreme Gradient Boosting) was trained on clinical data and data extracted from TTE reports to predict all-cause five-year mortality. The ML model was evaluated with data from a different tertiary center and against traditional risk scores as the reference standard.

Results: A total of 1253 patients (775 train set, 478 test set) were included, of which 176 patients (105 train set, 71 test set) died during the five-year follow-up period. The ML model demonstrated a superior performance (area under the curve [AUC] 0.79, Figure 1) compared to the Framingham risk (AUC 0.62) score, SCORE2/SCORE2-OP (AUC 0.67), a Cox-based model (AUC 0.76), and LV function (AUC 0.64). The ML model showed good external performance (AUC 0.78). Seven clinical variables and two TTE variables (left ventricular dysfunction and tricuspid regurgitation) were included in the final model.

Conclusion: This study showed that an ML model using TTE and clinical data can accurately identify high risk CCS patients, with a prognostic value superior to traditional risk scores.

Pitch + poster Abstract 9

Presenter: Mick Renkens

Atherosclerosis & Ischemic Syndromes

The Enduring Hiatus Between Clinical Guidelines and Clinical Practice in Appropriate Lipid-Lowering Therapy Still Affects a Third of Patients Undergoing PCI in Europe: Insights from PIONEER IV and Multivessel Talent Trials

Mick Renkens, MD; Joanna Wykrzykowska, MD, Ph.D ;Tsung-Ying Tsai, MD; Pruthvi C. Revaiah, MD; Shigetaka Kageyama, MD; Scot Garg, MD, Ph.D.; Robbert de Winter, MD, Ph.D.; Liesbeth Rosseel, MD; Edouard Benit, MD; Julien Lemoine, MD; Azfar Zaman, MD, Ph.D.; Adrian Wlodarczak, MD; Helge Mollmann, MD, Ph.D.; Manel Sabate, MD, Ph.D; Faisal Sharif, MD; Peter Doran, MD, Ph.D; Jagat Narula, MD, Ph.D; Wolfgang Koenig, MD, Ph.D.;Thomas F. Lüscher MD, FRCP; Yoshinobu Onuma, MD, Ph.D; Patrick W. Serruys, MD, Ph.D.

Cardiology Location AMC

Background: Coronary artery disease (CAD) remains a significant global health concern. In this study, we examined the current landscape of risk assessment and lipid-lowering therapy in patients undergoing PCI in Europe. The cornerstone in the pathogenesis of atherosclerosis is the penetration of the endothelial barrier by excessive small cholesterol particles, such as LDL-C and lipoprotein(a) where they interact with the local intimal tissue. For patients with established CAD, treatment focuses on two main goals: 1) aggressively controlling risk factors like cholesterol-containing lipoproteins to halt disease progression, and 2) preserving myocardial blood supply through pharmacotherapy or revascularization.

Methods: Our survey encompassed 2013 patients enrolled in international multicenter trials, PIONEER IV and Multivessel Talent, conducted across European PCI centers. Risk assessment and appropriateness of lipid-lowering therapy were reviewed by extracting the data from the trial databases. Conventional risk factors, lipid profile, inflammatory markers, and disease complexity were considered important. Lipid-lowering therapy regimes at 30-day follow-up were used to score appropriateness based on the estimated average effect of different drugs and combinations.

Results: Baseline biochemical profiles revealed that 50-60% of patients exhibited hsCRP levels >2mg/L. A 20% of patients had HbA1c >6.5% indicative of long-term hyperglycemia. 82% of patients (n=1430) had LDL-C values >1.4 mmol/L (55mg/dL). The target LDL-C of 1.4 mmol/L was unlikely to be reached in 34% (n=590). Statin usage increased from 80% to plateau around 90% at 30 days follow-up. Concurrently, Ezetimibe use gradually rose to 20-25%, whereas the adoption of PCSK9 inhibitors (and fibrates) remained <1%. Notably, there were minimal fluctuations in the proportion of lipid-lowering therapy used after the 30-day follow-up visit, indicating a lack of treatment optimization during follow-up.

Conclusions: In light of these findings, there is a gap to be bridged between current guideline recommendations and real-world practice. Importantly, reimbursement restrictions may severely limit appropriate lipid-lowering therapy.

Pitch + poster Abstract 10

Presenter: Alexandra Giovou

Heart Failure & Arrhythmias

Postnatal ventricular regeneration in congenital heart disease: function and application of TBX5 for promoting cardiomyocyte proliferation

Alexandra Giovou, Arnie Boender, Mathilde R. Rivaud, Gerard J. Boink, Monika M. Gladka, Vincent M. Christoffels

Medical Biology *Location AMC*

Congenital heart disease (CHD) refers to a range of structural malformations present at birth affecting the functionality of the heart. Our research focuses on the consequences of anomalies of the outflow track, the connection between the ventricles and the great arteries, which is formed during embryogenesis. Outflow track defects can lead to ventricular remodeling and dysfunction by altering the hemodynamic conditions. Despite the advances in the surgical field during infancy, young adults still risk developing pathological hypertrophy, cardiac arrhythmias, and heart failure. The patient outcome depends on multiple factors, including the state of the ventricular muscle at birth, impact of postnatal abnormal loading and residual dysfunction after surgical interventions.

In this project, we aim to increase the functionality of the congenitally malformed and stressed (right) ventricle, with our current main focus on restoring the cardiomyocyte (CM) number. In a previous established mouse model, we deleted the last intron of Tbx5 in order to study its dose-dependent effect on development and disease. Interestingly, we found that a slight elevation of ventricular Tbx5 expression before birth promotes proliferation of CMs.

Building on these results, we next wanted to study the role of TBX5 in early postnatal cardiac muscle proliferation and ultimately deliver TBX5 into a rat model mimicking congenital pulmonary artery stenosis and right ventricular failure, to test its potential as a therapeutic target.
In order to study the impact of TBX5 at the postnatal heart, we generated a cardiomyocyte-specific AAV9-hTNNT2-TBX5 vector and systemically delivered it to juvenile mice. Gene expression analysis (qPCR, RNAseq) revealed the upregulation of proliferative markers and downregulation of genes involved in energy metabolism, including fatty acid oxidation, a hallmark of de-differentiation. Immune fluorescent microscopy revealed that a small fraction of CMs incorporate EdU, indicative of cell cycle activity. Although TBX5 delivery was specific to CMs and expected to cell-autonomously induce cell cycle activity, co-localization of EdU and TBX5 was not detectable. Furthermore, transduced TBX5 differentially regulated the expression of its target genes such that some known targets (Gja5, Nppa) were induced, whereas others were unexpectedly down-regulated (Ryr2, Atp2a2), indicating that targets respond in a dose-dependent manner.

Our findings suggest that the CM-specific delivery of TBX5 promotes CM de-differentiation and cell-cycle activity, and differentially affects the responsiveness of key cardiac genes in a dose-dependent manner. In the near future, we will evaluate the impact of CM delivery of a lower dose of TBX5 on proliferation and gene regulation, and deliver TBX5 and other candidate pro-regenerative factors to models of CHD and cardiac injury to test its potential therapeutic application.

Pitch + poster Abstract 11

Presenter: Anoek Rooijakkers

Heart Failure & Arrhythmias

Long-range gene editing of LMNA

Anoek A.M.B. Rooijakkers, Yigal M. Pinto, Anke J. Tijsen

Experimental Cardiology *Location AMC*

Mutations in the LMNA gene commonly affect the cardiac muscle tissue, which leads to the development of a severe dilated cardiomyopathy with life-threatening arrhythmias. The lack of response to conventional heart failure therapy in these patients indicates the urgent need for new therapies. Gene editing has the potential to become a curative treatment for these patients by correcting the underlying genetic cause of the disease. Prime editing is a new gene editing technology, which uses a prime editing guide RNA (pegRNA) to target a specific genetic locus and creates edits using a reverse-transcriptase enzyme linked to a Cas9-nickase. With more than 600 disease-causing mutations described in LMNA, the feasibility of conventional prime editing, where one pegRNA targets one single mutation, is limited. Therefore, we aim to target not a single, but a set of mutations with one single pegRNA.

These pegRNAs are optimized using the fluoPEER reporter in HEK293T cells, which expresses mCherry following successful editing of the target mutation. Flow-cytometry analysis of treated cells revealed an editing efficiency of 12% with a long-range pegRNA, compared to 15% with a conventional short pegRNA targeting the same mutation <10bp downstream of the Cas9 nick site. This editing efficiency using the same long-range pegRNA was retained at 9% when editing at a distant position >100bp downstream of the nick site. To increase editing efficiency, we improved the stability of the pegRNAs by the addition of different 3’ structural motifs. This resulted in increased editing efficiencies of 15% and 17% for nearby and distant mutations, respectively.

In conclusion, we show that it is possible to edit different mutations within a range of up to 120 bp with a single pegRNA. Ongoing optimisations might further increase editing efficiencies and eventually provide a prime editing therapy able to cure disease in patients carrying different mutations in the same genetic region. For LMNA-associated diseases this would substantially reduce the number of pegRNAs that have to be developed to cover all mutations in the gene, greatly improving the feasibility of prime editing therapy.

Pitch + poster Abstract 12

Presenter: Thomas Steunenberg

Microcirculation

Clinical outcomes of 5 000 IU heparin versus activated clotting time guided heparinization during non-cardiac arterial procedures: a propensity score matched analysis

M. Hoebink, L.C. Roosendaal, M.J. Beverloo, A.M. Wiersema, T. van der Ploeg, T.A.H. Steunenberg, K.K. Yeung & V.Jongkind

Surgery *Location AMC*

Background: This study investigated clinical outcomes of 100 IU/kg heparin followed by activated clotting time (ACT)-guided heparinization in comparison to a standardized bolus of 5 000 IU heparin during non-cardiac arterial procedures (NCAP).

Methods: A retrospective cohort analysis from a prospectively collected database of patients undergoing NCAP in two vascular centers was performed. Patients receiving ACT guided heparinization were matched 1 : 1 with patients receiving 5 000 IU heparin, using propensity score matching (PSM). Primary outcomes were thrombo-embolic complications (TEC), mortality and bleeding complications within 30 days of procedure or during the same admission.

Results: 759 patients (5 000 IU heparin: 213 patients, ACT-guided heparinization: 546 patients) were included. PSM resulted in 209 patients per treatment group. After PSM groups were comparable, with the exception of an higher prevalence of peripheral arterial disease in the ACT-guided heparinization group ($p = .039$). Target ACT (> 200 s) was reached by 95% in the ACT group vs. 34% in the 5 000 IU group ($p < .001$). There was no significant difference between patients receiving ACT-guided heparinization and standard 5 000 IU heparin for TEC (6.2% vs. 4.8%, $p = .52$), mortality (1.4% vs. 0%) or bleeding complications (15% vs. 12%, $p = .32$). Multivariate logistic regression identified female sex, preoperative anaemia (men: < 8.1 mmol/L, women: < 7.5 mmol/L), preoperative kidney disease (eGFR < 60 ml/min) and open abdominal aortic aneurysm surgery as predictors for bleeding complications. Bleeding risk was lower after carotid surgery.

Conclusion: No difference in TEC, bleeding complications and mortality between ACT-guided heparinization and a single bolus of 5 000 IU heparin was found during NCAP.

Pitch + poster Abstract 13

Presenter: Carolien Volleman

Microcirculation

Endothelial activation in COVID-19 induced ARDS patients on ECMO support: a pilot study

Y. Li, C. Volleman*, A.M. de Boer, A.P.J. Vlaar, C.E. van den Brom, on behalf of the Amsterdam UMC COVID Biobank Investigators*
**Y. Li and C. Volleman contributed equally to this work*

Intensive Care Volwassenen Location AMC

Introduction: Extracorporeal membrane oxygenation (ECMO) is a last-resort therapy for critically ill patients with respiratory failure, including severe COVID-19-induced ARDS. However, ECMO has a high risk of complications. Such a complication is systemic inflammation and endothelial activation which remains poorly understood. This study aimed to assess longitudinal changes in markers of endothelial dysfunction in ECMO-supported COVID-19 patients and compare these markers to intubated COVID-19 patients.

Methods: Plasma was obtained from COVID-19 patients on ECMO support prior to ECMO, within 48 hours, on day 4, week 1 and week 2 of ECMO support. Intubated COVID-19 patients were used as controls and sampled once at ICU admission. Circulating markers of inflammation, endothelial dysfunction, glycocalyx shedding and hemostasis were measured using Luminex and ELISA.

Results: Between April 2020 and January 2022, samples were collected from 14 ECMO patients (71.4% male; age 53±9 years) and 28 intubated controls (78.6% male; age 54±9 years). ICAM-1 and E-selectin levels increased over time in ECMO patients (respectively 0.61 to 1.06 µg/mL, ptime=0.046; 30.99 to 43.90 ng/mL, ptime=0.001). Circulating angiopoietin-1 levels steadily decreased from 5.88 to 1.59 ng/ml (ptime<0.001), whereas levels of TNF-α, P-selectin, angiopoietin-2, syndecan-1, von Willebrand Factor and soluble trombomodulin remained stable. When compared to controls, patients eligible for ECMO showed increased levels of syndecan-1, von Willebrand Factor, and D-dimer (respectively 13.15 vs 9.06 ng/mL; p=0.015; 8.68 vs 6.77 ng/mL, p=0.037; 5.48 vs 3.72 µg/mL, p=0.024).

Conclusions: These pilot data suggest that ECMO support is associated with more endothelial activation, however, does not seem to further induce endothelial damage in severely ill patients. Moreover, patients eligible for ECMO have increased glycocalyx degradation prior to initiation when compared to controls. A prospective cohort study is necessary to elucidate whether these effects are specifically due to ECMO or the patients' disease progression.

Pitch + poster Abstract 14

Presenter: Beau Neep

Pulmonary Hypertension & Thrombosis

Understanding the role of SOX17 in the shear stress response of the arterial endothelium and its implication in early Pulmonary Arterial Hypertension development

Beau Fabienne Neep, Xiaoke Pan, Harm-Jan Bogaards, Jurjan Aman

Pulmonary Medicine Location VUmc

Pulmonary arterial hypertension (PAH) is a fatal disease. The loss of small pulmonary arterioles may contribute to PAH development. Recent genetic studies have identified SOX17 as novel regulator in PAH, where variations in SOX17 were shown to worsen the PAH phenotype and accelerate PAH development. SOX17 is a well-known embryonic arterial-specific transcription factor, and although its expression in adult arterial endothelial cells has also been confirmed with single-cell RNA sequencing of human lungs, its function in the adult vasculature is currently unknown. Based on previous genetic studies and pilot data, we hypothesized that SOX17 reinforces the arterial endothelium under shear stress, and that impaired SOX17 expression leads to loss of arterioles and contributes to PAH. When subjecting pulmonary artery endothelial cells (PAECs) to 72 hours of 15 dyn/cm2 shear stress, we indeed saw an increase in SOX17 expression on RNA and protein level. When we reduced the expression of SOX17 during shear stress using a lentiviral shRNA, we saw a decrease in the expression of various cell-cell adhesion markers, including VE-cadherin, NOTCH1 and tight junction proteins. We want to further investigate these downstream targets of SOX17 by performing a transcriptomic analyses of SOX17-depleted PAECs under shear. To study the shear stress pathway upstream of SOX17, we are currently investigating if knocking down shear receptors Endoglin and VEGFR2 alters SOX17 expression under shear. We have also shown that SOX17 expression can be increased by supplementing the cells with TGF-β pathway ligand BMP9, which indicates that SOX17 is (partially) controlled by the ALK1 receptor. Finally, we want to determine barrier permeability of SOX17-depleted PAECs under shear stress, since we have already observed a stronger increase in barrier permeability and recovery time after an inflammatory stimulus in the knockdown cells under static conditions.

Pitch + poster Abstract 15

Presenter: Sarah Hilderink

Heart Failure & Arrhythmias

Hypertrophic cardiomyopathy evident at an early age in knock-in mice with the most common Dutch founder mutation, MYBPC3c.2373InsG

Sarah Hilderink, Maike Schuldt, Max Goebel, Valentijn Jansen, Emmy Manders, Stan Moorman, Larissa M Dorsch, Frank G van Steenbeek, Jolanda van der Velden, Diederik WD Kuster

Physiology Location Vumc

Hypertrophic cardiomyopathy (HCM) is frequently caused by mutations in the cardiac myosin binding protein-C (cMyBP-C) encoding gene MYBPC3. In the Netherlands, approximately 25% of patients carry the MYBPC3c.2373InsG founder mutation. Most patients are heterozygous and have highly variable phenotypic expression, whereas homozygous patients have severe HCM at a young age. To improve understanding of disease progression and the genotype-phenotype relationship of HCM, we characterized young (3 week old) and adult (23 week old) mice by knocking-in the MYBPC3c.2373InsG mutation with CRISPR/Cas9. We assessed cardiac hypertrophy, non-sarcomere cytoskeletal profile, tubulin signature, and cardiomyocyte contractility in young and adult homozygous Mybpc3c.2373InsG mice (Mybpc3InsG/InsG), compared to age-matched wild-types. Knock-in of Mybpc3c.2373InsG successfully depleted cMyBP-C. At 3 weeks of age Mybpc3InsG/InsG hearts were already hypertrophied, as cardiac weight doubled compared to wild-types, which was also observed in adult Mybpc3InsG/InsG mice. Functionally, fractional shortening of young Mybpc3InsG/InsG cardiomyocytes decreased minimally, whereas it increased in the adult Mybpc3InsG/InsG cardiomyocytes. Contraction and relaxation rates were slowed in Mybpc3InsG/InsG cardiomyocytes, and to a greater extent in adult Mybpc3InsG/InsG cardiomyocytes. It is known that stiffened cytoskeleton and impaired relaxation are, in part, due to elevated levels of detyrosinated tubulin and desmin. This was evident in both young and adult Mybpc3InsG/InsG mice, and additionally, young Mybpc3InsG/InsG mice also had increased levels of acetylated-α-tubulin and tyrosinated tubulin, akin to obstructive HCM patients. Inhibiting tubulin detyrosination improved relaxation kinetics in adult Mybpc3InsG/InsG mice, indicating that pharmacological therapy is of benefit in Mybpc3c.2373InsG mutated cardiomyocytes. Moreover, the tubulin signature of 3 week old Mybpc3InsG/InsG mice resembles that of obstructive HCM patients, and 23 week old mice are representative of end-stage HCM with heart failure. Both the young and adult Mybpc3InsG/InsG models recapitulate HCM, with largely similar pathophysiology. Thus, pathophysiology of HCM in the Mybpc3c.2373InsG mouse model develops from an early age.

Pitch + poster Abstract 16

Presenter: Benthe Ariëns

Diabetes & Metabolism

The effect of SGLT2-inhibition on the kidney perfusion and diffusion in patients with T2D

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Internal Medicine & Radiology Location AMC

Introduction: Kidney hypoxia may be a key pathophysiological mechanism in the development of diabetic kidney disease in type 2 diabetes (T2D). Sodium-glucose cotransporter 2 (SGLT2) inhibitors such as Ertugliflozin are thought to be kidney protective agents, which may be related to (1) a decrease in hyperfiltration and (2) an alleviation of kidney hypoxia. To test this, Incoherent motion diffusion weighted imaging (IVIM-DWI) and phase contrast MRI allow for studying the flow and perfusion of the kidney. We hypothesize that Ertugliflozin has a positive effect on the alleviation of kidney hypoxia by decreasing hyperfiltration in the kidneys, measured by a decreased flow and perfusion.

Methods: In this single-center, double-blind crossover study, nineteen adults with T2D (90% male, mean age 64.9 ± 14.7 years) underwent four-week treatment with SGLT2 inhibitor ertugliflozin (ERTU) and matched placebo (PLB), followed by GFR assessment via iohexol clearance. Participants received abdominal imaging twice on a 3T MRI system (Philips), including DWI-IVIM and phase-contrast MRI. DWI-IVIM parameters were obtained using respiratory motion compensation and IVIMnetoptim neural network. Wilcoxon signed-rank tests compared SGLT2i and placebo effects.

Results: Flow and perfusion were significantly lower after four weeks of Ertugliflozin, corresponding to the measured lower GFR and could indicate a decreased hyperfiltration. Interestingly, an increase of D values was seen, suggesting a positive effect on the kidneys, as D values were seen to decrease as kidney fibrosis progresses in other studies.

Discussion: The protective effect of SGLT2-inhibition on the kidneys could result from a decrease hyperfiltration, which, in turn, can result from a lower flow, perfusion fraction and GFR. Furthermore, SGLT2-I increased diffusion, a value that decreases as renal insufficiency progresses.

Conclusion: In conclusion, SGLT2i reduces hyperfiltration in the T2D kidneys, lowering GFR and perfusion and arterial blood flow. Moreover, SGLT2i has a positive effect on the diffusion of the kidneys.

Pitch + poster Abstract 17

Presenter: Jordan Marcel Kraaijenhof

Atherosclerosis & Ischemic Syndromes

Targeting apolipoprotein C-III with olezarsen leads to significant reduction in fasted and postprandial triglyceride levels

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Vascular Medicine department *Location AMC*

Aims: Postprandial hypertriglyceridemia is an independent risk factor for atherosclerotic cardiovascular disease (ASCVD). Olezarsen is a novel N-acetyl-galactosamine-conjugated antisense oligonucleotide that targets apolipoprotein C-III (apoC-III) with the aim of lowering plasma triglyceride levels. This study investigates whether apoC-III inhibition mitigates the postprandial increase in triglycerides following a high-fat meal and whether the treatment affects monocyte phenotype, function, and plasmatic inflammatory biomarkers.

Methods and Results: We conducted a randomized, double-blind, placebo-controlled study in patients with hypertriglyceridemia, characterized by fasting triglyceride levels exceeding 4 mmol/L (350 mg/L). Patients were administered either two doses of 80 mg olezarsen or a placebo subcutaneously in a 2:1 ratio, separated by a 4-week interval. The primary endpoints were the percentage change in triglyceride area under the curve (AUC) levels and the change in monocyte expression markers compared to placebo after 7 weeks. A total of 31 patients were included, with a mean (standard deviation) age of 58.4 (±10.1) years, and 83.9% being male. The median (interquartile range [IQR]) baseline triglyceride level was 7.7 mmol/L (IQR: 4.2-9.2). Triglyceride levels increased from a fasted median of 7.7 mmol/L to 10.0 mmol/L four hours postprandially. Preliminary results show a significant reduction of 60.1% ($p<0.0001$) in baseline triglyceride levels compared to placebo. The triglyceride AUC was reduced by 48.9% ($p<0.0001$) compared to placebo, whereas the incremental AUC did not show a significant change (-9.6%, $p=0.52$).

Conclusion: In patients with moderate to severe hypertriglyceridemia, two doses of olezarsen significantly reduced both baseline and postprandial triglyceride levels. Subsequent analyses will provide further insight into the effects on circulating monocytes and plasmatic inflammatory biomarkers.

Pitch + poster Abstract 18

Presenter: Rushd F. M. Al-Shama

Heart Failure & Arrhythmias

Myeloperoxidase causes arrhythmogenic remodeling in cardiac slices

Rushd F. M. Al-Shama, Bashar K. Kahawati, Benedetta F. Fabrizi, Veronique Meijborg, Ruben Coronel, Bas J. Boukens, Joris R. de Groot

(Experimental) Cardiology Location AMC

Background: In atrial fibrillation (AF), neutrophils are activated, releasing myeloperoxidase (MPO) into the atrial tissue. MPO increases arrhythmia susceptibility and accumulates before AF onset. Increased serum MPO predicts treatment failure in AF patients. We sought to investigate the role of MPO in electrophysiological and structural remodeling using two different cardiac slice models: pig ventricular slices with homogenous architecture and a clinically relevant model of human atrial slices obtained from AF patients undergoing ablative surgery.

Methods: Viable slices with a thickness of 380 µm were generated using a vibrating microtome from pig ventricles and human left atrial appendages. The slices were then incubated with 1 µg/mL MPO or vehicle for 24 hours (ventricular slices: n=15/group, n=2 pigs; atrial slices: n=18 MPO, n=14 control, n=6 patients). Electrophysiological mapping was performed using an 8x8 multielectrode array (MEA). Action potentials (APs) were recorded using a microelectrode during pacing. Staining and qPCR were used to assess fibrosis markers and extracellular matrix gene expression, respectively.

Results: Compared to the control, ventricular slices incubated with MPO exhibited larger fractionation intervals and more heterogeneous conduction. Accordingly, TGFb1 was upregulated, and more collagen deposition and fibroblasts were present in these slices. Additionally, these slices showed a higher pacing threshold and slower conduction, with microelectrode recordings revealing a 10% depolarization of the resting membrane by MPO relative to controls. In MPO-treated atrial slices versus controls, a higher fractionation interval, more dispersion of long collagen fibers, and upregulation of COL1A1 and TIMP1 were observed. MPO similarly slowed conduction and depolarized the atrial slices by 33%, and more slices were spontaneously active relative to controls.

Conclusion: This study elucidates the role of MPO in arrhythmogenesis. By inducing depolarization, conduction slowing, and spontaneous activity, MPO creates a substrate favorable for re-entry. These findings suggest that MPO is a promising target for AF therapy.

Poster Abstract 19

Presenter: Sila Algül

Heart Failure & Arrhythmias

EGFR/IGF1R Signaling Modulates Relaxation in Hypertrophic Cardiomyopathy

Sila Algül, Maike Schuldt, Emmy Manders, Valentijn Jansen, Saskia Schlossarek, Richard de Goeij-de Haas, Alex A. Henneman, Sander R. Piersma, Connie R. Jimenez, Michelle Michels, Lucie Carrier, Michiel Helmes, Jolanda van der Velden and Diederik W.D. Kuster

Physiology Location Vumc

Background: Diastolic dysfunction is central to diseases such as heart failure with preserved ejection fraction and hypertrophic cardiomyopathy (HCM). However, therapies that improve cardiac relaxation are scarce, partly due to a limited understanding of modulators of cardiomyocyte relaxation. We hypothesized that cardiac relaxation is regulated by multiple unidentified proteins and that dysregulation of kinases contribute to impaired relaxation in HCM patients.

Methods: We optimized and increased the throughput of unloaded shortening measurements and screened a kinase inhibitor library in isolated adult cardiomyocytes from wild-type (WT) mice. 157 kinase inhibitors were screened. To assess which kinases are dysregulated in HCM patients and could contribute to impaired relaxation, we performed a tyrosine and global phosphoproteomics screen and Integrative Inferred Kinase Activity (INKA) analysis using HCM patient myocardium. Identified hits from these two dataset were validated in cardiomyocytes from a homozygous MYBPC3c.2373insG HCM mouse model.

Results: Screening of 157 kinase inhibitors in WT (n=33) cardiomyocytes (n=24,563) resulted in the identification of 17 positive inotropes and 21 positive lusitropes, almost all of them novel. The positive lusitropes formed three clusters: cell cycle, EGFR/IGF1R and a small Akt signaling cluster. By performing phosphoproteomic profiling of HCM patient myocardium (n=24 HCM, n=8 donors), we demonstrated increased activation of 6 out of 8 proteins from the EGFR/IGFR1 cluster in HCM. We validated compounds from this cluster in mouse HCM (n=12) cardiomyocytes (n=2,023). Three compounds from this cluster were able to improve relaxation in HCM cardiomyocytes.

Conclusion: We showed the feasibility of screening for functional modulators of cardiomyocyte relaxation and contraction, parameters that we observed to be modulated by kinases involved in EGFR/IGF1R, Akt, cell cycle signaling and FoxO signaling, respectively. Integrating the screening data with phosphoproteomics analysis in HCM patient tissue indicated that inhibition of EGFR/IGF1R signaling is a promising target for treating impaired relaxation in HCM.

Poster Abstract 20

Presenter: Rianne Baelde

Heart Failure & Arrhythmias

Kbtbd13R408C-knockin mouse model elucidates mitochondrial pathomechanism in NEM6

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Physiology Location Vumc

Nemaline Myopathy type 6 (NEM6) is characterized by muscle weakness and impaired relaxation kinetics and caused by variants in Kelch-repeat-and-BTB-(POZ)-Domain-Containing-13 (KBTBD13). The majority of the NEM6 patients harbors the Dutch founder mutation KBTBD13R408C (c.1222C>T, p.Arg408Cys). Histological characterization of NEM6 patient biopsies by NADH staining showed the presence of cores, indicating the absence of complex I (NADH) activity, suggesting mitochondrial dysfunction. Here, we aimed to investigate whether mitochondrial dysfunction contributes to NEM6 disease pathology. Therefore, we used the Kbtbd13R408C-knockin mouse model that phenocopies NEM6 hallmarks.

In vivo, homozygous Kbtbd13R408C-knockin mice display a significant impaired running performance, decreased VO2max and increased respiratory exchange ratio (RER) compared to wildtype (WT) mice. Additionally, in vitro mitochondrial respiration showed a significant decreased complex I (NADH) linked respiration and total oxidative phosphorylation in homozygous Kbtbd13R408C-knockin mice, indicating that mitochondrial dysfunction contributes to the lower VO2max and running performance assessed in vivo. Next, we performed enzymatic NADH stainings on cryosections of soleus muscle from Kbtbd13R408C-knockin and WT mice to assess enzymatic activity in both slow-twitch (type I) and intermediate/fast-twitch (type IIa) myofibers. Soleus muscle of Kbtbd13R408C-knockin mice showed cores in both type I and type IIa myofibers on NADH staining.

To conclude, the presence of cores in myofibers of Kbtbd13R408C-knockin mice phenocopies the presence of cores in muscle of NEM6 patients. The Kbtbd13R408C-knockin mouse model revealed that mitochondrial dysfunction contributes to NEM6 disease pathology. Next, we will use the Kbtbd13R408C-knockin mouse model to study the onset and progression of mitochondrial dysfunction in NEM6 and test interventions that target mitochondrial function.

Poster Abstract 21

Presenter: Rianne Baelde

Heart Failure & Arrhythmias

Kbtbd13R408C-knockin mouse model reveals impaired relaxation kinetics as novel pathomechanism for NEM6 cardiomyopathy

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Physiology Location Vumc

The mechanisms that modulate muscle relaxation kinetics are critically important for muscle function. A prime example of the impact of impaired relaxation kinetics is nemaline myopathy caused by variants in KBTBD13 (NEM6) encoding kelch repeat and BTB (POZ) domain containing 13. The majority of NEM6 patients harbors the Dutch founder variant, c.1222C>T, p.Arg408Cys (KBTBD13R408C). Recently, we discovered that the Dutch founder variant not only affects skeletal muscle function, but also results in cardiac dysfunction. The mechanism underlying cardiac dysfunction in NEM6 is completely unknown. Histological evaluation of cardiac structure in Kbtbd13R408C-knockin mouse model – a model that is well characterized and phenocopies NEM6 myopathy- revealed no abnormalities. Pressure-volume loop analyses showed the end-diastolic pressure-volume relation was steeper in Kbtbd13R408C-knockin mice, indicating diastolic dysfunction.

We aimed to study the contraction and relaxation kinetics in wild type (WT) and Kbtbd13R408C-knockin mice at the intact single cardiomyocyte level by a high-throughput contractility set-up. Our data showed an increased relaxation time in Kbtbd13R408C-knockin mice compared to WT mice, indicating impaired relaxation kinetics. No differences in the magnitude of contraction (percentage of shortening) was found between cardiomyocytes of Kbtbd13R408C-knockin and WT mice. In parallel, calcium-handling was assessed by calcium indicator Fura-2AM. These studies reveal that calcium-release is not affected, but calcium-reuptake is impaired in Kbtbd13R408C-knockin mice, which might contribute to the impaired relaxation kinetics.

Current studies focus on how KBTBD13 affects calcium-reuptake kinetics and sarcomere kinetics in NEM6 cardiomyocytes. Hence, our studies provide the first insights in the pathomechanism underlying cardiac dysfunction in NEM6.

Poster Abstract 22

Presenter: Fjodor Bekedam

Pulmonary Hypertension & Thrombosis

Cardiac fibroblasts in pulmonary arterial hypertension have altered mechanosensing

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3: Department of Cell and Chemical Biology, Leiden UMC

Pulmonary Medicine Location Vumc

Introduction: Pulmonary arterial hypertension (PAH) is a rare, fatal disease characterized by pulmonary vascular remodeling. Narrowing of the pulmonary arterioles leads to increased right ventricular pressure followed by fibrosis and failure. Here, the objective was to study the behavior of cardiac fibroblasts from PAH patients under mechanically active and profibrotic conditions.

Methods: iPSC were generated from healthy subjects and PAH patients. The iPSC were differentiated into cardiac fibroblasts (CF) over 20 days. First, iPSC were stimulated with 12 µM CHIR99021 for 24h, followed by 24 hours recovery and 18 days of FGF2 stimulation. The iPSC derived CF were characterized at the gene and protein level and compared to primary cells to verify their identity. A gel contraction assay was performed to study remodeling capacity and responsiveness to ml transforming growth factor β (TGFβ). Finally, the differentiated cells were exposed to 10 ng/ml TGFβ and 10% cyclic stretch at 1 Hz for 3 days using the Flexcell FX-6000 system.

Results: The differentiated cells had a fibroblast-like morphology. Furthermore, the presence of cardiac (GATA4, TCF21) and fibroblast (VIM, PDGFRα, DDR2) markers at gene and protein levels confirmed the CF identity. At the end of differentiation, here was no significant difference in any of the markers tested between primary as well as healthy and PAH iPSC derived cells. Stimulation with the profibrotic cytokine TGFβ resulted in an upregulation of fibroblast activation markers, such as α-smooth muscle actin (α-SMA). When exposed to cyclic stretching a downwards trend of fibroblast activation was observed in healthy cells, but not in PAH cells. Furthermore, cycling stretching was able to protect healthy cells from TGFβ mediated fibroblast activation. However, PAH cells did not benefit from the positive effects of stretching indicating that mechanotransduction of these cells may be impaired.

Poster Abstract 23

Presenter: Ruggero Belluomo

Heart Failure & Arrhythmias

Identifying angiogenesis-regulating lncRNAs to regenerate the damaged myocardium following Myocardial Infarction

R. Belluomo, V. Kremer, M. Bennet, AH. Baker, RP. Brandes, RA. Boon

Physiology Location Vumc

Myocardial Infarction (MI) is characterized by the sudden death of myocardial tissue due to ischemic damage leading to significant death of cardiac cells. To salvage dying cells, angiogenesis has been identified as a key therapeutic target to treat MI by restoring the blood flow in the infarcted area. Our aim is to identify novel lncRNA that regulate angiogenesis to enhance this process and rescue the dying heart. To do so, we investigated the gene response of human Cardiac Microvascular Endothelial Cells (CMECs), one of the most abundant cell types in the heart, to a human cardiac cell lysate. hCMECs were stimulated with cardiac lysate for 24h and then RNA sequencing, EdU assay, and wound-healing assay were performed. The RNA sequencing revealed a total of 1294 Differently Expressed Genes (DEGs) of which 130 were lncRNAs and the Gene Set Enrichment Analysis (GSEA) revealed that proliferation (mitosis), migration, and angiogenesis were among the most enriched terms. Moreover, Correlation analysis with a publicly available mouse MI model revealed that 25% of the DEGs were in common between the two datasets, and 70% shared the same up-/down-regulation indicating the relevance of this model in MI. The EdU essay and the wound/healing assay confirmed the GSEA and showed an increase of 12-30% (N=4 **) in proliferation and 56% in wound area coverage upon stimulation with cardiac lysate. We then found around 25 DE lncRNAs co-expressed with proteins involved in cell cycle and mitotic entry (Pearson 0.6-0.99 and p-value < 0.05) such as HIF1A-AS3, DNM3OS, HCP5, DLGAP1-AS2. Knockdown experiments for DLGAP1-AS2 and DNM3OS showed an increase of up to 36% (p 0.04, N=4) and 22% (p 0.07, N=4) in proliferation, and up to 70% and 50% in sprouting respectively. Knockdowns for HIF1a-AS3 and HCP5 still need optimization.

Poster Abstract 24

Presenter: Sean Benson

Heart Failure & Arrhythmias

Digital twins for enhanced multi-modal predictions in clinical data

Dr Sean Benson, Prof. Dr Folkert Asselbergs

Cardiology Location AMC

Digital twins are broadly defined as models of complex systems, based increasingly on multi-modal data, that provide the ability to simulate future events or interventions.The creation of such models requires that datasets be large enough to properly characterise the statistical relationships between the different features upon which the twin is built. However, when fine-tuning models to new cohorts, decreasing performance on the original training dataset is a known issue. This issue is difficult to solve when access to the original training dataset is not possible. We describe the use of graph deep learning digital twin models in order to generate synthetic multi-modal datasets from the Lifelines study of 170,000 citizent. The synthetic datasets are then used in combination with real hospital patient data for the training of a downstream classification model to predict accute cardiac events based on multiple clinical sources including demographic, lifestyle, and ECG data.

Poster Abstract 25

Presenter: Daan Bosshardt

Atherosclerosis & Ischemic Syndromes

4D aortic motion of Marfan syndrome patients derived from 3T bSSFP CMR

D. Bosshardt, R. Merton, A.J. Nederveen, D. Robbers-Visser, E.M. Schrauben, M. Groenink, P. van Ooij

Radiology and Nuclear Medicine Location AMC

Background: Aortic diameter is the only biomarker for aortopathy in Marfan Syndrome (MFS). Abnormal distensibility and motion of the aorta may also play a role in aortic growth and dissection. We investigated 4D aortic motion as a potential new biomarker for aortic disease in MFS using 4D bSSFP CMR.

Methods: 8 MFS patients (5 females, aged 27 ± 2.83 years, 6 with a history of aortic root surgery) and 4 age- and gender-matched controls (3 females, age 26±2.83 years) underwent imaging of the thoracic aorta using a non-contrast enhanced, free breathing, time-resolved three dimensional (3D)CINE bSSFP sequence on an Ingenia 3T MRI scanner1. PROspective Undersampling in multiple Dimensions was used for acceleration resulting in R~10 (scan time ~4 minutes)2. Scan parameters were FOV=256×256×88mm3; acquired/reconstructed spatiotemporal resolution = 1.6/1.0mm3, ~67ms (15 cardiac phases, (CP)), TR/TE/FA=2.9ms/1.44ms/40°. A nnU-Net was used to automatically segment all CP3. The time-resolved segmentations were used to derive 4D aortic diameter maps4. Displacement and diameter change of the ascending aorta (AAo) were derived using an iterative closest point registration of a single reference end-diastolic phase to all CP4, 5. CP were grouped for early-, peak and late-systole and diastole.

Results: AAo diameter was significantly larger for MFS patients versus controls in diastole. Change in AAo diameter was significantly smaller for MFS patients versus controls during peak- and late-systole and diastole and displacement was smaller for MFS patients in peak- and late-systole. In MFS patients with a replaced aortic root we found a larger diameter (mm) in early-systole and diastole and smaller displacement in peak-systole and diastole compared to patients without root surgery.

Conclusion: MFS patients had lower diameter, diameter change and less displacement of the aorta during the cardiac cycle compared to healthy controls. These findings may be useful for the monitoring of aortic disease in MFS.

Poster Abstract 26

Presenter: Thomas Bouwmeester

Atherosclerosis & Ischemic Syndromes

Altered neurocardiac regulation predicts new-onset hypertension and systolic blood pressure increase in a multi ethnic cohort: The HELIUS study

T. A. Bouwmeester, D. Collard, E. M. C. Vriend, H. Galenkamp, B. E. Westerhof, B. J. H. van den Born

Vasculaire Geneeskunde Location AMC

Introduction: Cross-correlation baroreceptor sensitivity (xBRS) and heart rate variability (HRV) provide valuable insights into neurocardiac regulation on a broad scale. However, their prospective utility in predicting new-onset hypertension and increases in systolic blood pressure (SBP) over time within a general population has not yet been demonstrated.

Methods: Data was collected from participants of the HEalthy LIfe in an Urban Setting (HELIUS) study. A non-invasive continuous finger BP-measurement at baseline was used to calculate xBRS, standard deviation of normal-to-normal interval (SDNN) and the squared root of mean squared successive differences between normal-to-normal intervals (RMSDD). In the normotensive individuals at baseline, we used logistic regression models to calculate the odds ratio (OR) of developing hypertension at follow-up, and in the total cohort we assessed the increase in SBP between baseline and follow-up. All analyses were performed in a crude model (adjusted for age, sex, ethnicity and SBP at baseline) and second model (further adjusted for smoking status, the presence of diabetes mellitus and BMI). A sensitivity analysis was performed in the younger (<50 y) and older (≥ 50 y) part of the population.

Results: Data from 4,910 participants were analyzed. A 50% reduction in xBRS is associated with an increased OR of 1.46 (95%CI 1.22–1.74) for developing hypertension in the total cohort, with no significant difference between the younger and older participants. A 50% reduction in RMSDD was associated with an increased OR for developing hypertension of 1.21 (95%CI 1.03-1.43), no significant association between SDNN and new-onset hypertension was found. A 50% lower xBRS, SDNN and RMSDD were associated with a higher delta SBP of 1.70, 0.92 and 0.81 respectively, mainly driven by the younger participants.

Conclusion: In the general population, a higher xBRS is associated with increased odds of developing hypertension, and a higher increase in SBP over time.

Poster Abstract 27

Presenter: Tessa Brik

Heart Failure & Arrhythmias

Patients' experiences with AF screening in primary care and the impact of receiving abnormal ambulatory ECG results – a qualitative sub-study of the PATCH-AF trial

Brik T, Niekel MS, Himmelreich JCL, Harskamp RE, Moll van Charante EP

General Practice Location AMC

Background: The European Society of Cardiology recommends systematic AF screening in high risk patients. Before implementing appropriate risk-based AF screening at a national healthcare level, it is crucial to fully grasp the pros and cons of screening. However, little is known about the patients' experience in AF screening and the impact that being diagnosed with AF or non-AF incidental findings has on their health-related quality of life.

Objective: This study aims to explore patients' experience with AF screening and how they perceive and cope with abnormal ambulatory ECG results, including AF or other incidental findings, and its impact on their health-related quality of life.

Methods: We performed a qualitative study, using semi-structured interviews with a thematic approach. Participants were purposively sampled from the PATCH-AF screening trial set in Amsterdam primary care, in which we focused on those who received an abnormal ambulatory ECG result.

Results: We conducted 14 interviews until data saturation was reached. Participants were 77.3 ±6.8 years old, 54% male, and 15% from a low socioeconomic background. Patients' experiences with abnormal AF screening results are influenced by factors like their expectations, the screening process, diagnosis comprehension, and subsequent diagnostic workup and treatment. Positive experiences include feeling reassured by healthcare providers and benefitting from early diagnosis before symptoms or complications emerged. Conversely, negative experiences involve diminished well-being due to unexpected diagnoses, distrust in the diagnosis and uncertainty about future prognosis or complications. Non-AF incidental findings often led to lack of clarity about the diagnosis and treatment.

Conclusion: This study identifies both positive and negative experiences in receiving abnormal ambulatory ECG results during AF screening. Understanding these is vital for tailoring screening programs and enhancing patient support throughout the process. Furthermore, it shows that more clarity about incidental ECG findings detected during AF screening is warranted.

Poster Abstract 28

Presenter: Rocco Caliandro

Atherosclerosis & Ischemic Syndromes; Heart Failure & Arrhythmias

Developing living myocardial tissue slices as model for studying cardioprotection after ischemic injury

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Medical Biology *Location AMC*

Background: Despite scientific advances in disease modelling, cardiac regenerative medicine lacks models that recapitulate the adult heart's complexity and mature phenotype. Hence, the availability of novel technologies would greatly benefit researchers investigating cardiac diseases. We use adult pig hearts to produce viable organotypic tissue slices that can be cultured for a prolonged time and can be manipulated to model diseases and interventions. Unlike other non-in vivo models, myocardial tissue slices recapitulate the architecture and complexity of the adult heart while being cost- and time-effective.

Methods: Left ventricular cardiac slices were generated from adult pigs' hearts and kept in culture for up to 4 days. We tested several treatments, including H2O2-induced stress or viral-mediated gene delivery.

Results: We optimized the slicing procedure to acquire 300 um-thick left ventricular slices. The electrical function and viability of the myocardial slices were assessed by performing pseudo-ECG or optical mapping. Slices could be locally paced up to a few days after slicing. Next, the cultured slices were treated with H2O2. After treatment, changes in the expression of stress marker and apoptosis marker genes were quantified. Next, we tested adeno-associated viruses (AAV)-mediated gene transfer efficiency to our model. Four days after the infection, AAV6 and AAV9 serotypes caused robust transduction and transgene expression of the cardiac slices. Finally, we achieved AAV6-mediated delivery of Zinc Finger E-Box Binding Homeobox 2 (ZEB2), a transcription factor involved in endothelial-mesenchymal transition that has been shown to promote cardiac repair after injury stimulating the secretion of pro-angiogenetic factors. Four days after infection, slices were collected for downstream analyses to further elucidate how ZEB2 stimulates cardioprotection in the heart.

Conclusions: Adult left ventricular tissue slices represent a suitable model for studying the pathophysiology of the adult heart and an alternative to in vivo cardiac disease models currently available.

Poster Abstract 29

Presenter: Simona Casini

Heart Failure & Arrhythmias

Impact of microtubule detyrosination on Nav1.5 channel function and subcellular distribution in mdx cardiomyocytes

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Experimental Cardiology *Location AMC*

Background: Subcellular trafficking and distribution of the cardiac sodium channel Nav1.5 in cardiomyocytes (CMs) is dependent on the microtubule (MT) network as well as Nav1.5 interacting proteins. Duchenne muscular dystrophy (DMD) is associated with loss of dystrophin, a Nav1.5-interacting protein localized at the lateral membrane (LM) of CMs, leading to sodium current (INa) reduction in this microdomain. Cardiomyocytes of DMD (mdx) mice also display increased MT detyrosination, but its impact on INa and Nav1.5 distribution is unclear.

Purpose: To investigate the effect of reducing MT detyrosination by the compound parthenolide (PTL) on INa and subcellular Nav1.5 distribution in mdx CMs.

Methods and Results: Cardiomyocytes from wild type (WT) and mdx (DMD) mice were incubated with either 10 µM PTL or DMSO for 3-5 hours. INa properties were assessed using the patch-clamp technique, confocal microscopy was used to investigate microtubule detyrosination, and stochastic optical reconstruction microscopy (STORM) was employed to assess Nav1.5 cluster density and size at the cardiomyocyte LM and intercalated disc (ID). Compared to WT, mdx CMs displayed increased levels of detyrosinated tubulin and decreased INa. Incubation with PTL decreased the fraction of detyrosinated tubulin and significantly increased INa in mdx CMs, but had no effect in WT CMs. Finally, STORM analysis showed that PTL increased Nav1.5 cluster density at both LM and ID in mdx CMs while it had no effect on WT CMs.

Conclusions: In mdx CMs, reduction of MT detyrosination rescues INa and increases Nav1.5 cluster density both at the ID and LM. Hence, in addition to loss of dystrophin, MT remodeling contributes to INa reduction in DMD. The PTL-induced increase in Nav1.5 at the LM of mdx CMs is likely due to a dystrophin-independent mechanism since dystrophin is normally absent from this microdomain. Overall, our findings identify MT detyrosination as a potential therapeutic target for modulating INa and subcellular Nav1.5 distribution during pathophysiological conditions.

Poster Abstract 30

Presenter: Margaux Cé

Pulmonary Hypertension & Thrombosis

Developing an iPS-atrial cardiomyocyte model expressing BMP10

M. Cé, A. Llucià-Valldeperas, R. Smal, M. J. Goumans, G. Sánchez-Duffhues, F. Handoko-de Man

Pulmonology *Location Vumc*

Pulmonary arterial hypertension (PAH) is a rare and fatal disease characterized by the remodelling of pulmonary vasculature leading to an increase in afterload, resulting in right heart adaptation and eventually right heart failure. Genetic mutations in the bone morphogenetic protein (BMP) pathway are frequently observed in PAH patients. The major predisposing risk factor in PH is loss-of-function mutations in the BMP receptor 2 (BMPR2) gene for which soluble BMP10 is its ligand. Our pilot data indicates that BMP10 activity and expression is increased in PAH-patients. Currently, the only PAH model using human induced pluripotent stem cells (hiPSC) is of ventricular cardiomyocytes. However, In the adult heart, BMP10 is produced in the right atrium. Therefore, to understanding how BMP10 expression is regulated in cardiomyocytes and the role it plays in the development of PAH, we are developing an atrial human induced pluripotent stem cells cardiomyocytes (hiPS-CM) model expressing BMP10. In order to develop this model, different concentrations and timepoints of retinoic acid exposure were used to obtain atrial cardiomyocytes. To increase the purity of the cardiomyocytes, metabolic and manual selections were both. In a recent paper, Ordono et al report an increase of BMP10 expression in ventricular hiPS-CM following lactic acid administration. Following their protocol, atrial hiPS-CM have been treated with lactic acid to study its effect on BMP10 expression. Atrial and ventricular cardiomyocytes were both characterized by their gene and protein expression, and beating patterns. BMP10 expression will be measured through an activity assay with immortalized endothelial green fluorescence protein lines, that will emit fluorescence when BMP10 binds to BMPR2.

Poster Abstract 31

Presenter: Lucas Celant

Pulmonary Hypertension & Thrombosis

Minimal important difference of NT-proBNP in pulmonary arterial hypertension

L.R. Celant, L.J. Meijboom, H.J. Bogaard, F.S. de Man and A. Vonk Noordegraaf

Pulmonary Medicine Location Vumc

Background: In pulmonary arterial hypertension (PAH), N-terminal pro B-type natriuretic peptide (NT-proBNP) reflects right ventricular structure and function. Elevated levels indicate increased wall stress and have shown to harbor prognostic information. In clinical trials, NT-proBNP is therefore often utilized as secondary endpoint, but the magnitude of a relevant change is unknown.

Research question: What is a relevant change of NTproBNP in PAH?

Methods: Incident, treatment naïve PAH-patients with available NT-proBNP and cardiac magnetic resonance (CMR) imaging at baseline and follow up were included. Stroke volume (SV) was used as anchor to establish the minimal important difference (MID) of NT-proBNP. Patients were divided in two groups based on their treatment response:1) patients with an increase in SV of at least 10 mL; 2) patients whose SV remained stable or did not increase beyond 10mL. Statistical approaches included: the optimal cutoff point on receiver operating curve (ROC) analysis, mean change in NT-proBNP between groups and bivariate linear regression to correct for covariates.

Results: Upon treatment, 52 patients had an increase in stroke volume of at least 10mL (SV≥10 patients) compared to 83 who failed to do so (SV<10 patients). Groups were similar in terms of gender (70% female), age (53yrs) and disease severity (PVR: 930 [604, 1182] vs. 633 [453, 903] dynes.sec.cm-5). Naturally, SV≥10 patients had a greater increase in SV (17 ml/m2; 26±8 to 43±10 ml/m2) compared to SV<10 patients (2 ml/m2; 32±9 to 34±9 ml/m2). NT-proBNP levels at baseline were greater for SV≥10 patients (1529 [432, 2847] vs. 603 [182, 1829] pg/ml). Overall, the MID range between methods is small and were as follows: ROC (-78%), mean change difference (-81%) and bivariate linear regression adjusted for age and gender (-79%).

Conclusion: The MID of NT-proBNP to identify patients with an increase of at least 10mL in SV was approximately -80% using anchor-based approaches.

Poster Abstract 32

Presenter: Aina Cervera i Barea

Heart Failure & Arrhythmias; Atherosclerosis & Ischemic Syndromes

AAV6-mediated gene transfer of HCN1-DDD generates slightly faster baseline beating rates as compared to Hcn2 yet with a potential risk for pro-arrhythmia

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Medical Biology *Location AMC*

Electronic pacing is the current treatment of choice for complete atrioventricular block (CAVB) that revolutionized last century's standard of care. Despite its success, some critical issues remain such as providing suboptimal cardiac output and a lack of direct autonomic responsiveness. To develop a more physiological and hardware-free pacemaker, we focused on developing gene therapy-based biological pacemakers. The end goal aimed at assessing the long-term biological pacemaker efficacy of AAV6-mediated gene transfer of an engineered mutant version of HCN1-DDDt and the wild-type Hcn2.

Functional biological pacemaker studies were conducted in a porcine model of radiofrequency ablation-induced CAVB. Four weeks after the ablation, animals were studied in two different immunosuppressed groups: AAV6-cTnT-HCN1-DDDt and AAV6-cTnT-Hcn2. All animals were then followed for another four weeks to evaluate in vivo biological pacemaker performance and then organs were harvested to assess transduction efficiency.

One week after transduction, beating rates rapidly increased trending down until basal pacing rhythms were reached. Transient increase in maximal HR was also detected compared to pre-transduction stages, where HCN1-DDDt offered the most potent and sustained response, despite rhythms were above an ideal physiological range. Finally, a remarkable reduction in electronically paced beats of roughly 50% was registered 1w post-gene transfer coinciding with a biological pacemaker phenotype that gradually rose above 80%. Nonetheless, 4w post-transduction HCN1-DDDt animals showed a significant reduction in backup pacing compared to Hcn2 suggesting a most favourable biological pacemaker phenotype long-term.

In conclusion, AAV6-mediated HCN1-DDDt gene transfer produced slightly faster mean heart rates as compared to Hcn2, yet HCN1-DDDt generated persistently increased in maximal heart rates (in two out of three animals) that exceeded the physiologically desirable range. These findings possibly relate to the much faster channel gating kinetics of HCN1-DDDt that were previously found to be profoundly pro-arrhythmic with the chimera channel HCN212.

Poster Abstract 33

Presenter: Wout Claassen

Pulmonary Hypertension & Thrombosis

Transcriptional activity of individual myonuclei in the atrophic diaphragm of mechanically ventilated ICU patients

W.J. Claassen, A. Bamyani, T. Kirby, L. Heunks, C. Ottenheijm

Physiology Location Vumc

Introduction: ICU-acquired diaphragm weakness affects more than half of mechanically ventilated ICU patients and may be caused by ventilator over or under assistance, resulting in atrophy and weakness of diaphragm muscle fibers. In diaphragm muscle undergoing atrophy, protein synthesis rates may decrease. We recently observed a decrease in myonuclear content in atrophic fibers isolated from diaphragm biopsies of mechanically ventilated ICU patients. This raises the question whether transcriptional activity of the remaining nuclei in the atrophic muscle fiber remains static during atrophy. In this study we investigate whether transcriptional activity is diminished in mechanically ventilated ICU patients.

Methods: Diaphragm biopsies of mechanically ventilated ICU patients (45h) with established atrophy (n=5) were compared to biopsies of patients who underwent thoracic surgery for a small, primary, pulmonary nodule (n=5) (Controls). We determined the transcriptional activity of single nuclei within manually isolated single muscle fibers by staining the enzyme responsible for transcription (RNA-polymerase-II) with an antibody specific for a post-translational phosphorylation at Serine 5. This phosphorylation occurs when the enzyme is activated and transcription of DNA into mRNA starts. Fluorescence intensity of phosphorylated RNA-polymerase-II within each nucleus was quantified in 3D.

Results: Total fluorescence of phospho-RNA-Poll-II C-terminal domain (Ser5) staining within individual nuclei from ICU patients (2235) and control patients (2817) was measured. There were no significant differences in transcriptional activity per nucleus between the groups of 401 [222-638] in the ICU group vs 428 [246-615] in the control group arbitrary units(AU), p=0.89 (median [IQR]). Furthermore, transcriptional activity normalized to fiber volume was not different across both groups.

Conclusion: These findings indicate that transcriptional activity is not diminished within the atrophic diaphragm of mechanically ventilated ICU patients. This finding does not support the hypothesis that diminished transcriptional output contributes to early diaphragm atrophy in mechanically ventilated patients.

Poster Abstract 34

Presenter: Amelie Collinet

Heart Failure & Arrhythmias

Dissecting the Molecular Mechanism of Atrial Fibrillation

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Physiology Location Vumc

Atrial Fibrillation (AF) is a tachyarrhythmia affecting atrial cardiomyocytes. Aberrant electrical impulses overpower the conduction initiated by the heart's natural pacemakers. As untreated AF may severely reduce the quality of live and can have fatal consequences like stroke and heart failure, reliable treatment is essential. Despite being the most common western cardiac tachyarrhythmia, treatment options remain insufficient due to high prevalence of AF episode recurrence. Since mechanisms underlying electropathology are still vastly unknown, the development of targeted pharmacotherapy remains challenging. AF can be triggered by environmental or physiological factors. Exposure to increased histamine levels or long-term stress can induce AF. In this project, the regarding driving mechanisms are to be dissected. Therefore, DNA damage, mitochondrial dysfunction and cytoskeletal integrity are investigated upon trigger-induction, alongside cardiomyocytes functionality. In order to prevent animal-based experiments, numerous validated AF-models including cell culture, human IPSC-derived atrial cardiomyocytes and Drosophila will be used. Finally, findings will be tested regarding their potential as biomarkers or even treatment target by pharmaceutical or genetic intervention. Early diagnosis as well as a reliable, long-term treatment method are essential to improve clinical outcome of AF patients, just as their quality of life.

Poster Abstract 35

Presenter: Josine de Winter

Heart Failure & Arrhythmias

KBTBD13 is a novel cardiomyopathy gene

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Physiology Location Vumc

KBTBD13 variants cause nemaline myopathy type 6 (NEM6). The majority of NEM6 patients harbors the Dutch founder variant, c.1222C>T, p.Arg408Cys (KBTBD13 p.R408C). Although KBTBD13 is expressed in cardiac muscle, cardiac involvement in NEM6 is unknown.

Here, we constructed pedigrees of three families with the KBTBD13 p.R408C variant. In sixty-five evaluated patients, 12% presented with LV dilatation, 29% with LVEF<50%, 8% with atrial fibrillation, 9% with ventricular tachycardia and 20% with repolarization abnormalities. Five patients received an implantable cardioverter defibrillator, three cases of sudden cardiac death were reported. Linkage analysis confirmed co-segregation of the KBTBD13 p.R408C variant with the cardiac phenotype. Mouse studies revealed that (1) mice harboring the Kbtbd13 p.R408C variant display mild diastolic dysfunction; (2) Kbtbd13-deficient mice have systolic dysfunction.

Hence, (1) KBTBD13 is associated with cardiac dysfunction and cardiomyopathy; (2) KBTBD13 should be added to the cardiomyopathy gene panel; (3) NEM6 patients should be referred to the cardiologist.

Poster Abstract 36

Presenter: Meagan Doppegieter

Microcirculation

Pulsed Dye laser treatment of psoriasis is based on perivascular nerve remission

Meagan Doppegieter, Nick van der Beek, Leah Wilk, Maurice Aalders, Ton van Leeuwen, Erik N.T.P. Bakker

Biomedical Engineering & Physics Location AMC

Introduction: Psoriasis is characterized by an increase in the proliferation of endothelial cells, resulting in an increased amount of tortuous vasculature in the papillary dermis of the skin. Pulsed Dye Laser (PDL) treatment targets the red blood cells and leads to hyperthermia of the blood vessels. PDL was initially developed to treat port wine stains, but has also been shown effective for treatment of psoriasis, although its mechanism remains unclear. One hypothesis is that PDL causes thermal damage by the diffusion of heat, generated in the vasculature, on neighboring structures in the skin like perivascular nerves and keratinocytes. There is limited information on the specific thermal sensitivity of these neighboring skin cells when exposed to hyperthermia. Our study investigated the effects of sub-minute exposure times and mild hyperthermia (45-70°C) to cell type specific damage both in vitro and ex vivo. Through our continuous research, we aim to validate the hypothesis that hyperthermia causes nerve damage. At the ACS symposium, we will show our findings from various research methodologies, including in-vitro, ex-vivo, in-vivo, and in-silico studies.

Materials & Methods:
In vitro: We studied the thermal sensitivity of keratinocytes, endothelial, smooth muscle, and neuronal cells through exposure to hyperthermia (45–70 °C) for various time points (2-20 sec) after which viability was measured.
Ex-vivo: Using wire myography, we tested three essential cell types in blood vessels: endothelial, smooth muscle cells, and vascular nerves. Blood vessels were exposed to hyperthermia (45–65°C) and cell-specific functionality was assessed before and after hyperthermia.
In vivo: We obtained pre- and post-PDL treatment biopsies from psoriasis patients and conducted staining for tissue innervation, vascularization, and immune cells. Advanced imaging software generated 3D skin reconstructions that were also used in in-silico modeling of heat distribution in the skin during PDL (work by Leah Wilk).

Results: Both our in-vitro and ex-vivo data show that cell damage occurs, and blood vessel functionality decrease after exposure to 55-60°C, and neuronal cells and keratinocytes are more susceptible to hyperthermia than blood vessel-specific cells such as endothelial cells. Our in-silico modeling shows that heat dissipates to the perivascular spaces, reaching temperatures of 55°C and higher . Analysis of in-vivo biopsy data is underway and preliminary data can be presented.

Conclusion: We have strong evidence that supports our hypothesis that PDL treatment heats not only the blood vessels but also causes thermal damage to the perivascular spaces where nerves reside. This understanding is crucial for the optimization and use of PDL treatment in the future.

Poster Abstract 37

Presenter: Inez Duursma

Heart Failure & Arrhythmias

Microtubule-Induced Restriction of Nuclear Deformability in Hypertrophic Cardiomyopathy

Inez Duursma, E.E Nollet, V. Jansen, K. Bedi, K.B. Margulies, N.N. van der Wel, J. van der Velden, T.J. Kirby, D.W.D Kuster

Physiology Location Vumc

Hypertrophic cardiomyopathy (HCM) is a disease characterized by abnormal thickening of the left ventricular wall and diastolic dysfunction. Cytoskeletal remodeling has been observed in HCM patients, with a robust increase in tubulin and desmin levels, which contributes to diastolic dysfunction and tissue stiffness. The pathophysiology of HCM is not fully understood, but the hypothesis is that misregulated mechanotransduction plays an essential role in the disease process.

This study aimed to relate cytoskeleton changes to nuclear and chromatin morphology in HCM patient myocardium and assess nuclear deformability in contracting cardiomyocytes with and without microtubule destabilizing drugs. Nuclear morphology and chromatin organization were assessed using electron microscopy in cardiac septal tissue from patients with obstructive HCM and compared to samples from non-failing donors. Additionally, nuclear changes were studied using wild type (WT) and homozygous Mybpc3c.2373InsG mice (Mybpc3c.2373InsG, KI), which have an HCM phenotype.

Cardiomyocyte nuclei of HCM patients had a significantly higher number of invaginations (0.32±0.09 invaginations/µm in HCM versus 0.18±0.03 invaginations/µm in NF hearts), were more irregular in shape, and had altered chromatin organization compared to those from non-failing hearts. Nuclei of Mybpc3c.2373InsG mice were considerably larger (322±75 μm^3 versus 221±24 μm^3) and less deformable than those of wild type mice. Nuclear deformability could be restored to wild-type levels by using microtubule-modifying drugs that decrease microtubule detyrosination. Additionally, nuclei from the HCM mouse model had more extensive DNA damage (19.7±3.4 foci in Mybpc3c.2373InsG versus 9.1±3.2 foci in WT).

In conclusion, the study demonstrated that cytoskeletal remodeling in HCM leads to less deformable nuclei, which may contribute to chromatin alterations and increased DNA damage. Nuclear deformability can be restored by microtubule modifying drugs. These findings provide new insights into the role of the cytoskeleton in the pathophysiology of HCM and suggest potential therapeutic targets for the disease.

Poster Abstract 38

Presenter: Mitchell Fiet

Atherosclerosis & Ischemic Syndromes

Systemic inflammation enhances mast cell infiltration in coronary arteries in patients who died from myocardial infarction

M.D. Fiet; W.W. Fuijkschot; L. Woudstra; R. van der Linden; D. Beskers; I.P.A. Zethof; S. Simsek; P.A.J. Krijnen; H.W.M. Niessen; Y.M. Smulders

Pathology Location AMC

Objective: Risk of cardiovascular events peak during and following systemic inflammation, such as infection. The acuteness of this increased risk suggests intra-plaque inflammation and subsequent plaque instability. We hypothesized that acute inflammation (AI) induces such changes and addressed this hypothesis in an autopsy study of patients with acute myocardial infarction (AMI) and with or without infection at time of death.

Methods: We selected patients with (n=18) and without (n=16) non-cardiac AI at time of death. Groups were divided by time since infarction (<6 hours vs. 6 hours to 14 days), as duration may influence plaque inflammation. Plaque characteristics were assessed in coronary sections. Densities of lymphocytes, macrophages, mast cells and neutrophilic granulocytes in the intima, media and adventitia were assessed.

Results: In 289 coronary artery sections, we found no difference in plaque characteristics or density of most inflammatory cells between AMI patients with or without AI. Mast cells, however, were increased in the intima (3.84 vs 2.42 cells/mm²)(P=0.022) and media (2.15 vs 0.83 cells/mm²)(P<0.0001) of early AMI patients with AI compared to the group without AI. Lymphocyte and macrophage density increased with advancing infarct duration, with no clear difference between the AI and no-AI groups.

Conclusions: AI may enhance mast cell influx in the intima and media of coronary arteries and contribute to the pathogenesis of AMI. Our results argue against a role for other inflammatory cells. Inflammatory plaque changes occurring later in the post-MI period are probably not influenced by the presence of AI.

Poster Abstract 39

Presenter: Nico Hahn

Atherosclerosis & Ischemic Syndromes

Lipids, the Achilles' heel of inflammatory macrophages?

Nico Hahn, Laszlo Groh, Daan Heister, Jan van den Bossche

MCBI Location Vumc

Inflammation plays a central role in the development of cardiovascular disease (CVDs) and is mediated by an invasion of immune cells into arteries that propagate a state of chronic inflammation. Within atherosclerotic plaques, macrophages are skewed towards an inflammatory state by environmental stimuli. Modulating inflammatory macrophage activation has a huge potential for the treatment of CVDs. As a key controller of macrophages, cellular metabolism is the focus in the development of new therapies. Here, we developed a 96-well-plate-based metabolic screening assay to discover new drugs with the potential to treat inflammatory diseases and adjust the balance between inflammatory and anti-inflammatory macrophages. This revealed FATP2, a long chain fatty acid transporter, as a unique therapeutic target in inflammatory macrophages. Inhibition of FATP2 caused a significant downregulation of the metabolic activity and accelerated cell death in LPS-activated macrophages. In addition, depletion of specific fatty acids prevented the metabolic switch towards glycolysis and reduced the generation of ROS that is triggered by LPS. These metabolic dependencies could be only observed in inflammatory macrophages, and not in naïve or IL-4-stimulated macrophages, highlighting a crucial role for long chain fatty acids in inflammatory macrophages. The aim of this study is to reveal the metabolic pathways explaining the dependency of inflammatory macrophages on lipids and to pinpoint the particular fatty acids underpinning this state. Unraveling this metabolic vulnerability in inflammatory macrophages could provide new strategies to combat CVDs.

Poster Abstract 40

Presenter: Katie Hanford

Atherosclerosis & Ischemic Syndromes; Microcirculation

A Novel 3-D Plaque-on-chip model to investigate endothelial-plaque microenvironment crosstalk in atherosclerosis

Katie M.L. Hanford, Kim E. Dzobo, Miranda Versloot, Jeffrey Kroon

Experimental Vascular Medicine Location AMC

Despite the introduction of novel lipid-lowering strategies many patients with cardiovascular disease continue to suffer from residual inflammation. Therefore, the identification of new therapeutic targets is necessary. Recapitulation of the in vivo situation is paramount when searching for new targets. It is therefore important to develop models that closely mimic the in vivo environment.

We hypothesized that low-grade residual inflammation (IL-1β) mediates vascular endothelial inflammation via metabolic rewiring, thereby sustaining a pro-inflammatory atherogenic environment. In this project, we aim to create a 3D plaque-on-a-chip model to mimic atherosclerosis in an in vitro setting. Additionally, by modulating endothelial cell metabolism under low-grade inflammatory conditions we aim to influence the subendothelial atherogenic plaque microenvironment.

We utilised the Emulate organ-on-a-chip technology to develop a plaque-on-a-chip model, consisting of a blood vessel compartment and a plaque-micro-environment. Currently, we are introducing a low-dose inflammatory stimulus (TNFα) under flow. We will investigate the effect of inflammation on endothelium and the subsequent cross-talk with the plaque environment on macrophage polarisation. Future studies will investigate whether macrophage polarisation is attenuated following modulation of metabolic pathways in the endothelium.

In addition, this model can also be created entirely using patient material to create a patient-tailored plaque-on-a-chip model. This is of particular interest to study the functional consequences of genetic metabolic defects on endothelial cell function and CVD risk. Furthermore, this system can be utilised to test therapeutics in a step to increase the translational ability of preclinical in vitro work.

Poster Abstract 41

Presenter: Philipp Hauger

Microcirculation

VoC model to study endothelial barrier function and polarity

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Physiology *Location Vumc*

Endothelial cells form a single layer of cells that forms the innermost layer of blood vessels. As such, they form a semi – permeable barrier between the blood on the apical side, and surrounding tissue on the basal side [1]. To prevent pathological endothelial dysfunction, the switch between stable and instable barrier has to be tightly regulated. In this regard, two major systems are at play: I) Molecular signaling cascades in endothelial cells (for example via small Rho-GTPases), that facilitate the strength of cell-cell contacts between endothelial cells; and II) multicellular crosstalk with cells that surround endothelial cells basally, namely vascular smooth muscle cells (VSMCs) and pericytes. As a result of their physiological environment, endothelial cells are exposed to distinct signals from the apical blood flow, and basal surrounding tissue (VSMCs and Pericytes) [2, 3].

Our group has recently defined novel regulators of cell-cell and cell-matrix adhesion in healthy and diseased endothelial cells. We aim to show that these regulators are vital to maintain endothelial cell polarity and barrier integrity. We will approach this question in conventional 2D cell models and more complex 3D Vessel on Chip systems (VoC), that include ECs, VSMCs and/or pericytes to study endothelial polarity in a highly translatable environment. To further increase physiological relevance, we will work with iPSC derived cells. This approach allows to generate isogenic multicellular VoCs and opens up the opportunity to include patient derived cell lines that carry mutations known to perturb endothelial polarity. This project aims to identify novel key players that maintain endothelial apico-basal polarity, which is valuable information in a translational context for CVDs that show impaired EC polarity.

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Poster Abstract 42

Presenter: Wenjun He

Pulmonary Hypertension & Thrombosis; Microcirculation

Unveiling the Impact of VEGFR2/ KDR Modulation on Pulmonary Microvascular Endothelial Cells in COPD/Emphysema

Wenjun He, Xiaoke Pan, Xiaoqing Sun, Harm Jan Bogaard, Jurjan Aman

Pulmonary Medicine Location Vumc

Background: Chronic obstructive pulmonary disease (COPD) is characterized by a rising global prevalence and an increasing disease burden, yet its precise pathophysiological mechanisms remain elusive. Recent research has unveiled loss of the pulmonary microvasculature as important contributor to emphysema. As the vascular endothelial growth factor receptor 2 (VEGFR2) is involved in vascular homeostasis, we hypothesize that VEGFR2 importantly determines lung microvascular integrity.

Aim: The aim of this study is to investigate the effects of vascular endothelial growth factor receptor 2 (VEGFR2) inhibitors SU5416 and shKDR on MVECs in the context of COPD /emphysema.

Methods: MVECs were treated with SU5416, DMSO (control), and pLKO (control) or shKDR constructs, and exposed to shear stress. Morphological changes in MVECs, including cell number, gap size and quantity, alignment, and elongation, were evaluated using confocal microscopy. Additionally, we explored how shear stress modulates cell proliferation via VEGFR2 downstream signalling pathways. Cell proliferation was assessed in shKDR-treated MVECs using MTT, Ki67, and wound healing assays.

Results: In MVECs shear stress induced a time-dependent increase in VEGFR2 Tyr1175/ERK signaling, but not Tyr951/Akt signaling. In addition, a time-dependent upregulation of total VEGFR2 and VE-cadherin was found, indicating that VEGFR2 acts as shear sensor and may stabilize the barrier under shear. Treatment with the VEGFR2 inhibitor SU5416 and shKDR resulted in perturbations in cell alignment and elongation. In addition, a decrease in the MVECs cell number and an increase in gap size and quantity was observed.

Conclusions: These findings underscore the significance of VEGFR2 in maintaining the structural and functional integrity of MVECs, specifically under shear stress. Perturbations in VEGFR2 signaling (in particular Tyr1175/ERK) signaling may contribute to microvascular instability, further emphasizing the critical role of VEGFR2 in pulmonary vascular health.

Keywords: Chronic obstructive pulmonary disease, VEGFR2, KDR, microvascular endothelial cells, Vascular integrity

Poster Abstract 43

Presenter: Aukie Hooglugt

Microcirculation

DLC1 promotes mechanotransductive feedback for YAP via RhoGAP-mediated focal adhesion turnover

Aukie Hooglugt1,2,#, Miesje M. van der Stoel1,#, Apeksha Shapeti3, Beau F. Neep1,4, Annett de Haan1, Hans van Oosterwyck3,5, Reinier A. Boon2,6,7 & Stephan Huveneers1

Medical Biochemistry *Location AMC*

Angiogenesis is a highly regulated process that requires a delicate balance between pro-angiogenic signals and vascular stabilizing factors. Nuclear translocation of the transcriptional co-factors YAP/TAZ upon substrate stiffening and VEGF stimuli plays a crucial role in driving migratory transcriptional programs in angiogenic endothelial cells. In addition, feedback mechanisms are needed to prevent excessive sprouting and ensure efficient collective endothelial migration and angiogenesis. The focal adhesion-related protein Deleted-in-liver-cancer-1 (DLC1) was recently described as a prominent transcriptional downstream target of YAP/TAZ in endothelial cells. In this study, we uncovered a negative feedback loop between DLC1 expression and YAP activity during collective migration and sprouting angiogenesis. Specifically, we demonstrated that signaling through the RhoGAP domain of DLC1 decreases the nuclear localization and transcriptional activity of YAP. Moreover, the RhoGAP function of DLC1 is needed for YAP-driven cellular processes, including focal adhesion turnover, proper mediation of traction forces and sprouting angiogenesis. Our findings show that DLC1 limits intracellular contractility through the inhibition of Rho/ROCK signaling, which in turn attenuates nuclear YAP localization. Altogether, these results place DLC1 expression and consequent reduced intracellular tension as a crucial mechanotransductive feedback event to modulate YAP activity during sprouting angiogenesis.

Poster Abstract 44

Presenter: Fabries Huiskes

Heart Failure & Arrhythmias

RNA-sequencing Approach to Understanding Atrial Fibrillation Electropathology

Fabries G. Huiskes, Esther E. Creemers, Yannick J. H. J. Taverne, Natasja M. S. de Groot, Bianca J. J. M. Brundel

Physiology Location Vumc

Background: Despite many efforts to treat atrial fibrillation (AF), the most common progressive and age-related cardiac tachyarrhythmia in the Western world, the efficacy is still suboptimal. Improved treatments could be directed at underlying molecular root causes that drive electrical conduction disorders and AF (i.e., electropathology). Insights into AF-induced transcriptomic alterations may aid in a deeper understanding of electropathology. Specifically, RNA sequencing (RNA-seq) facilitates transcriptomic analyses and discovery of differences in gene expression profiles between patient groups. In the last decade, various RNA-seq studies have been conducted in atrial tissue samples of patients with AF versus controls in sinus rhythm. We aim to contrast published and new RNA-seq data to uncover root causes of AF.

Methods and Results: We have reviewed 17 recent RNA-seq studies to provide an overview of the available human AF RNA-seq studies and highlight the molecular pathways identified. Also, a comparison is made between human RNA seq findings with findings from experimental AF model systems. Previous published data identified differentially expressed molecular pathways, including mechanotransduction, ECM remodeling, ion channel signaling, and structural tissue organization through developmental and inflammatory signaling pathways. Similar pathways were observed in large animal models of AF. However, there was very little overlap in specific DEGs when comparing the two most recent large cohort AF patient studies, as well as when comparing with two large animal model studies. Variation in underlying heart disease within and between patient groups hinders comparisons between studies.

Conclusions: Informed by the difficulties in comparing the reviewed studies to find specific molecular root causes of AF, we propose a transcriptomics study that combines deep RNA-seq with detailed electrophysiological mapping of the patient's atria, in carefully matched SR and AF cohorts. The combined analysis of electrical conduction disorders and molecular mechanisms in AF is a potent progression in uncovering the electropathology of AF.

Poster Abstract 45

Presenter: Lily Jalink

Microcirculation

Unraveling the role of long non-coding RNA AC024909.1 in heart failure with preserved ejection fraction

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Physiology Location Vumc

Background: Heart failure with preserved ejection fraction (HFpEF) affects the majority of HF patients. HFpEF is associated with a systemic pro-inflammatory state induced by comorbidities and aging, causing myocardial structural and functional alterations. However, the underlying molecular mechanism is not fully understood, leading to a lack of effective treatments. Endothelial dysfunction is known to be involved in HFpEF and our previous studies have shown the direct regulatory effect of endothelial cells on cardiac muscle function. The function of endothelial cells is regulated by long non-coding RNAs (lncRNAs), a group of non-protein-coding transcripts larger than 200 nucleotides. lncRNAs display diverse cellular functions and are implicated in cardiovascular pathophysiology. However, their role in HFpEF is not well known. We have discovered that lncRNA AC024909.1 is downregulated in HFpEF myocardial biopsies. In this study, we aim to further elucidate the role of AC024909.1 in HFpEF.

Methods and Results: Our in silico analysis on RNA sequencing data from cardiac biopsies of HFpEF patients shows reduced expression of AC024909.1 (~2-fold, $p<0.0001$), and expression was reduced upon senescence of hCMECs, suggesting that AC024909.1 may mediate endothelial dysfunction-driven pathogenesis of HFpEF. Knockdown of AC024909.1 in hCMECS co-cultured with rat cardiomyocytes reduced the contractile function of cardiomyocytes. In silico analysis predicts an ORF in the sequence of AC024909.1, suggesting that it might code for a micropeptide.

Conclusion and Outlook: Our preliminary data show that AC024909.1 is downregulated in HFpEF and is important for CMEC functionality. Silencing AC024909.1 in CMECs leads to impaired endothelial-enhancement of cardiomyocyte function. However, its mechanism of action is to be further elucidated. We expect that this lncRNA functions as a endothelial-derived micropeptide that regulates cardiomyocyte function. To elucidate the molecular mechanism of AC024909.1, we will use lentivirus overexpression and mass spectrometry technologies. AC024909.1 may serve as a potential druggable target for the treatment HFpEF.

Poster Abstract 46

Presenter: Carolina Janssen Telders

Heart Failure & Arrhythmias

What does computer tomography tell us about epicardial adipose tissue in heart failure with preserved ejection fraction?

Carolina Janssen Telders, E.R. Meulendijks, J.R. de Groot, L.J. Meijboom, F.S. de Man, M.L. Handoko

Cardiology Location VUmc

Background: Epicardial adipose tissue (EAT) is thought to significantly contribute to the pathophysiology of heart failure with preserved ejection fraction (HFpEF) through mechanical compression and/or lipotoxicity. With computed tomography (CT), volume and attenuation (as a surrogate measure of inflammation) of EAT can be quantified.

Aim: To investigate the relation of EAT volume and attenuation as measured by CT with HFpEF probability (H2FPEF-score).

Methods: 81 cardiac patients from the Amsterdam UMC underwent (non-)contrast CT-imaging. Scans were analysed through machine learning software (QFAT, V.9.5) that allowed for semi-automated quantification of EAT volume (cm3) and attenuation (HU). The H2FPEF score (0-9 points) categorized participants into low/intermediate (<6) versus high (≥ 6 points) probability of having HFpEF. EAT volume was corrected for body surface area (BSA). Data are presented as mean ± SEM. EAT volume and attenuation were compared between both categories using the parametric student t-tests, with a significant level set at $p<0.05$.

Results: Of all participants, 45 (56%) had a low/intermediate probability and 36 (44%) had a high probability for HFpEF (age: 64±7 years, females: 38%). Those with a high probability had a significant higher corrected EAT volume than those with a low/intermediate probability for HFpEF (Figure 1a; 63.0±3.6 vs 52.1±2.6 cm3/m2; $p<0.05$). Regarding EAT attenuation, participants with a high probability for HFpEF had a (unexpectedly) lower attenuation than participants with a low-intermediate probability (Figure 1b; -78.2±0.9 vs. -74.7±0.6 HU; $p<0.05$), suggestive of less inflammation.

Conclusion: This study demonstrates that a high HFpEF probability is associated with greater total EAT volume. However, it is associated with lower inflammatory activity, indicated by a lower attenuation. EAT tissue analyses will be performed to further investigate the underlying pathophysiology of EAT in HFpEF patients.

Poster Abstract 47

Presenter: Zhu Jiang

Microcirculation

Intramyocardial blood vessels have increased pro-fibrotic cellular transition and increased perivascular fibrosis in myocardial infarction

Zhu Jiang MSc,, Hans W.M. Niessen MD PhD,, Paul A.J. Krijnen PhD

Pathology Location AMC

Background: Intramyocardial vascular inflammation and dysfunction are believed to increase the risk of myocardial infarction (MI). Perivascular fibrosis and pro-fibrotic cellular transitions have been shown to be important predictors of cardiovascular disease in general. In this study, we studied intramyocardial blood vessels in deceased patients during the early and chronic phases of MI to explore the involvement of these factors in the pathological process of MI.

Methods: Left ventricles (LV) were obtained from autopsied patients in the early phases of MI (3-6 hours) (n=23), the chronic phase of MI (n=12) (5-14 days), and control subjects (n=14). We analyzed perivascular fibrosis using EVG staining and FAP expression using immunohistochemistry. Immunofluorescent co-localization of SMA with S100A4 or CD31 with S100A4 served as markers of pro-fibrotic cellular transition in intramyocardial vessels.

Results: Compared to controls (61.03% [12.60%]), perivascular fibrosis was significantly elevated in MI patients (early phase 77.20 [9.02]; chronic phase 82.58 [5.16]), with the highest level observed in the infarction area of chronic phase (95.18 [4.41]). The expression of FAP in the LV myocardium of early-phase MI patients exhibited a 3.5-fold increase compared to controls, while chronic-phase MI patients showed a 15.3-fold increase, with the infarction area being approximately three times larger than the non-infarction area. The fraction of SMA+S100A4+ intramyocardial blood vessels and the fraction of CD31+S100A4+ area were significantly higher in early-phase MI patients compared to controls.

Conclusion: We observed increased perivascular fibrosis and pro-fibrotic cellular transition in intramyocardial blood vessels in MI patients, suggesting that phenotype switching in vascular cells, fibroblast activation, and the extent of perivascular fibrosis contribute to the development of MI.

Poster Abstract 48

Presenter: Timo Jonker

Heart Failure & Arrhythmias

Strategies for transcriptional optimization of cardiac gene therapy

T. Jonker; A.R. Boender; M. Klerk; L. Kwakman; C.M.L Nelissen; P. Barnett; V.M. Christoffels; G.J.J. Boink

Medical Biology *Location AMC*

Gene therapy for cardiovascular diseases has seen several clinical trials, among others aimed at heart failure. However, these trials have not had satisfactory outcomes, despite encouraging pre-clinical outcomes. The challenges in cardiac gene therapy development have been attributed to several factors, from transgene ineffectiveness to immune responses and low expression. These hurdles call for optimization of all aspects of gene therapy interventions. In this project, our aim is to optimize the promoter of cardiac gene therapy vectors beyond the current state of the art.

Based on RNA-seq and ATAC-seq, we selected candidate promoter regions from a set of highly expressed and accessible cardiac genes, and have tested these in vitro in a cardiomyocyte-like cell line using luciferase assays. A selected set was produced as AAV9 luciferase reporter vectors, which were systemically injected at 5*10^11 vg/mouse. Luciferase expression was quantified in tissue homogenates of the left ventricle, right ventricle, atria and liver.

In vitro screenings revealed 8 novel promoter candidates with significantly higher expression in the cardiomyocyte-like cell line compared to benchmark TNNT2 promoter, while all were significantly weaker compared to the non-tissue-specific CMV promoter. Subsequent in vivo injection of an initial subset of 6 novel promoter candidates, together with benchmark CMV and TNNT2 promoters, revealed none of the novel candidates was stronger, nor more specific than benchmark TNNT2 promoter. Additionally, we found vector build-up in the liver compared to other organs, together with leaky expression in liver from TNNT2. These off-target effects may necessitate further optimization of capsid and promoter of cardiac-targeted gene therapy vectors. Current work is focused on further optimizing the promoters identified in this screen and testing new promoter design strategies. Comparative analysis of in vitro assays in the cardiomyocyte-like cell line and neonatal rat ventricular myocytes also showed the latter faithfully predicted in vivo mouse activity.

Poster Abstract 49

Presenter: Rio Juni

Heart Failure & Arrhythmias; Microcirculation

Novel endothelial-enriched long non-coding RNA IRENE is dysregulated in HFpEF and regulates endothelial enhancement of cardiomyocyte function

Rio P. Juni, Philippa Phelp, Lily Jalink, Denise Busscher, Ruggero Belluomo, Veerle Kremer, Anke van Bergen, Reinier A. Boon

Physiology Location Vumc

Background: Heart failure with preserved ejection fraction (HFpEF) represents the largest unmet clinical need in cardiovascular medicine. HFpEF is characterized by cardiac microvascular dysfunction. We have shown that cardiac endothelium regulate cardiomyocyte relaxation, which is impaired when endothelial cells are dysfunctional. Long non-coding RNAs (lncRNAs) have been implicated in various cardiovascular diseases. However, their role in HFpEF is unknown. We discovered a novel lncRNA AL590004.3 (IRENE), which is reduced in HFpEF patients. We hypothesize that IRENE is pivotal in endothelial dysfunction-driven pathogenesis of HFpEF.

Methods and results: IRENE is significantly downregulated (~7 fold, $p<0.001$) in RNA sequencing analysis of cardiac biopsies from HFpEF patients. RNA sequencing comparing various human cell types found the highest expression of IRENE in cardiac microvascular endothelial cells (CMECs), indicating its importance in cardiac endothelial function. IRENE was reduced upon CMEC exposure to pro-inflammatory cytokines TNFα (~2.5 fold, $p<0.05$) and IL1β (~2 fold, $p<0.01$), and upon senescent (2 fold, $p<0.005$), indicating its responsiveness to inflammation and ageing, two prevalent risk factors in HFpEF. IRENE is mostly expressed in the nucleus (~90%), where the majority found within the chromatin, suggesting its function in transcription regulation. To elucidate its function, we assessed endothelial enhancement of cardiomyocyte function upon IRENE silencing in CMECs. Silencing of IRENE impaired endothelial function to enhance cardiomyocyte relaxation (~1.5 fold, $p<0.01$). We performed RNA sequencing on CMECs after IRENE silencing and revealed several promising differentially regulated genes that will be further investigated to dissect the mechanism of action.

Conclusion: We showed that IRENE is a cardiac endothelial cell-enriched lncRNA that is significantly downregulated in the heart of HFpEF patients. IRENE is important for cardiac endothelial cell function as loss-of-function in CMECs impaired endothelial enhancement of cardiomyocyte relaxation. Herewith, we name this lncRNA Important for the Regulation of ENdothelial function in HFpEF (IRENE).

Poster Abstract 50

Presenter: Kaj Kappe

Microcirculation

Outcomes of the Viabahn Balloon-Expandable Endoprosthesis as Bridging Stent-Graft for Fenestrated- and Branched Endovascular Aortic Repair

K.O. Kappe, MSc., S.E.M. van Knippenberg, MD*, B.L. Tran, BSc., R.J. Lely, MD, B.B. van der Meijs, MD, J.D. Blankensteijn, MD, PhD, R. Balm, MD, PhD, J.H. Nederhoed, MD, PhD, V. Jongkind, MD, PhD, A.W.J. Hoksbergen, MD, PhD, K.K. Yeung, MD, PhD*

Vascular Surgery Location VUmc

Introduction: Bridging stent-grafts implanted during fenestrated and branched endovascular aortic repair (F/B-EVAR) are crucial for the successful exclusion of the aortic aneurysm. The aim of this study was to analyze the outcomes of the Gore Viabahn VBX stent-graft as bridging stent for renal and visceral target vessels during F/B-EVAR.

Methods: We collected data of all consecutive patients undergoing F/B-EVAR that were treated with at least one VBX stent-graft as a bridging stent in the Amsterdam University Medical Centers from January 2019 to May 2023. Patients were treated for thoraco-abdominal or complex abdominal aortic aneurysms. The procedural, radiological and follow-up data of the included patients were retrospectively reviewed. Primary outcome of the study was technical success for VBX stent-graft implantation, defined as successful catheterization, placement and deployment of the VBX stent-graft in the intended target vessels. Furthermore, VBX-related endoleaks, stenoses and occlusions during follow-up were reported.

Results: A total of 274 VBX stent-grafts were implanted for 263 target vessels in 38 FEVAR, 46 BEVAR and 3 F/B-EVAR (combined design) stent-grafts in 87 patients (75% male; mean age, 73 ± 7 years). Technical success of VBX stent-graft implantation was 97.5% (273 out of 280 implantation attempts). Post-operative radiological evaluation of 239 VBX stent-grafts was available for analysis. The patency of the VBX stent-grafts was 98.3% (235/239) after a median follow-up of 12 months. During follow-up, VBX-related adverse events that required re-intervention occurred in 2.9% (7/239): two type 1c endoleaks, two type 3c endoleaks, two in-stent stenoses and one occlusion.

Conclusion: VBX stent-graft implantation as a bridging stent during F/BEVAR has a high technical success and show excellent patency as bridging stent-grafts in F/BEVAR with a low number of complications requiring re-intervention. Long term follow-up data are awaited.

Poster Abstract 51

Presenter: Micky Karsten

Diabetes & Metabolism

Peritoneal Dialysis (PD) Effluent Derived Extracellular Vesicles to Establish PD-induced Peritoneal Alterations

Micky Karsten, Michiel Pegtel, Johan de Rooij, Nils Groenewegen, Marc Vervloet, Lily Jakulj

Nephrology Location AMC

In peritoneal dialysis (PD) exposure of the peritoneal membrane to glucose ultimately results in membrane function loss and fibrosis. With evolving therapies, biomarkers to assess peritoneal vitality and response to interventions mitigating peritoneal injury are mandatory. Extracellular vesicles (EVs) have been investigated as easy-accessible and stable biomarkers. We describe a clinically applicable technique to isolate and analyse the molecular cargo of PD-effluent (PDE)-derived EVs (PDE-EVs).

PDE was collected from PD-treated adults. Cell-free PDE was obtained by centrifugation. PDE-EVs were isolated by subsequent filtration and size-exclusion chromatography (SEC). We used Western blot with EV-markers to confirm the presence of EVs in the SEC-fractions; qPCR of miRNA-21 and -10b was performed to check robustness of isolation. The molecular cargo of the PDE-EVs was analysed with miRNA sequencing. To explore whether different miRNA profiles were seen with a PD-vintage of less than 1 and more than 2 years, we performed a differential expression analysis.

PDE of 20 patients was collected after use of varying glucose concentrations, dwell-times and with or without icodextrin. We confirmed presence of PDE-EVs in the SEC-fractions by Western blot. Ct-values of miRNA-21 and -10b showed robust signals for all types of PDE. miRNA sequencing was of good quality with 400-700 different miRNAs per sample, which is comparable to plasma-EV sequencing. Despite the small sample size, differential expression analysis showed significantly higher values of miRNA-449a and -449c-5p in patients with a PD-vintage of more than 2 years. These miRNAs promote epithelial and endothelial to mesenchymal transition.

We present a reproducible and clinically applicable method to isolate and molecularly characterize PDE-EVs, with promising preliminary results on miRNA signature profiling. Further characterization of the molecular cargo of PDE-EVs may serve as a novel means to monitor peritoneal changes and as a potential biomarker for risk stratification in terms of PD-related clinical outcomes.

Poster Abstract 52

Presenter: Rosalie Kempkes

Atherosclerosis & Ischemic Syndromes

EZH2 inhibition reduces macrophage inflammatory responses in atherosclerosis

Rosalie W.M. Kempkes, Lea C.M. Rief, Cindy P.A.A. Roomen, Guillermo R. Griffith, Winnie G. Vos, Laura A. Bosmans, Marten A. Hoeksema, Marion J.J. Gijbels, Koen H.M. Prange, Menno P.J. de Winther, Annette E. Neele

Medical Biochemistry *Location AMC*

Aim: Epigenetic processes are essential modulators of macrophage inflammatory responses. We postulate that interference in the epigenetic machinery of macrophages might offer novel approaches to combat atherosclerosis. Here, we investigate the repressive histone modification H3K27Me3 deposited by the polycomb repressor complex 2 (PRC2) with its catalytic component EZH2. We studied the therapeutic potential of macrophage EZH2 inhibition in the context of atherosclerosis.

Methods: Human monocyte-derived macrophages and murine peritoneal and bone-marrow derived macrophages were treated with the EZH2-specific inhibitor GSK126 and subsequently activated with LPS to mimic TLR4-inflammatory responses. The impact of GSK126 on macrophage differentiation and activation compared to vehicle (DMSO) was assessed by RNA-seq, ChIP-seq, flow cytometry, western blot, and ELISA. Additionally, female Ldlr-/- mice on a high-fat diet for five weeks were treated for four weeks in addition to the diet by every other day intraperitoneal injections with GSK126 or control and assessed for the impact on atherosclerosis and immune cells.

Results: GSK126 treatment in vitro lowered global H3K27Me3 levels without altering macrophage viability and differentiation, showing effective EZH2 inhibition. RNA-seq revealed that of more than one-third of the LPS-induced genes were significantly downregulated by GSK126 treatment. Subsequent pathway analysis identified cytokine and interferon signaling, co-stimulation, and cell migration as the top down-regulated pathways (padj<0.05). Indeed, we confirmed that gene and cytokine expression of the inflammatory mediators IL-6, IL-12, and TNF were reduced. Furthermore, membrane marker expression of co-stimulatory CD40, CD80, and CD86 were significantly decreased. In murine atherosclerosis, we observed a reduction in the Virmani plaque severity score and are currently assessing lesion size and composition.

Conclusion: Overall, we show that EZH2 inhibition reduces inflammatory responses in human and murine macrophages in vitro. We are currently assessing the impact of EZH2 inhibition on lesion size and composition in murine atherosclerosis. Furthermore, we are performing ex vivo experiments on human endarterectomy plaques to assess the therapeutic potential of EZH2 inhibition on human atherosclerosis.

Poster Abstract 53

Presenter: Moeed Khokhar

Microcirculation

Neurovascular changes during and after ischemic stroke

Moeed Khokhar, I.A Mulder, E.T van Bavel

Biomedical Engineering and Physics Location AMC

With the use of thrombectomy as clinical intervention for acute ischemic stroke patients, brain-wide microvascular dysfunction and impaired reperfusion as contributors to secondary brain injury has become the main focus point. With this project we gain a better understanding of different processes responsible for this secondary brain injury.

We aim to investigate changes in neurovascular function during and after ischemic stroke using the transient Middle Cerebral Artery Occlusion (MCAO) model to mimic large vessel occlusion and recanalization in mice. Combined with a chronic cranial window and in vivo multi-photon microscopic imaging, we evaluate the neurovascular (dys)function and microcirculation over time in the acute and chronic phase after stroke onset and upon reperfusion. More specifically, we aim to investigate the role of pericytes and vasospasm, as well as the formation of de novo micro-thrombi and their influence on impaired reperfusion and secondary brain injury. With the use of transgenic mice we can visualize and study pericytes to gain a better understanding of the role these cells play in regulating blood flow during and after ischemic stroke.

This project will provide insights into the mechanisms underlying impaired reperfusion and secondary brain injury due to stroke-induced microvascular changes and possibly contribute to the development of new treatments for ischemic stroke patients.

Poster Abstract 54

Presenter: Amber Korn

Diabetes & Metabolism;Microcirculation

Stem cell therapy with a clinically relevant dose does not significantly affect atherosclerotic plaque characteristics in a type 1 diabetic mouse model

A. Korn, S. Simsek, M.D. Fiet, I.S.E. Waas, J.W.M Niessen, P.A.J. Krijnen

Pathology Location AMC

Background: Diabetes mellitus (DM) induces increased inflammation and calcification of atherosclerotic plaques, resulting in elevated plaque instability. Mesenchymal stem cell (MSC) therapy was shown to decrease plaque size and stability in non-DM animal models. We now studied the effect of MSC therapy in a streptozotocin-induced DM mouse model using a clinically relevant dose of adipose tissue-derived MSCs (ASCs).

Method: DM was induced in male C57/Bl6 ApoE-/- mice (n=24) via intraperitoneal streptozotocin (STZ) injection (0.05 mg/g bodyweight) for 5 consecutive days. 16 weeks after the first STZ injection, the diabetic mice received either 125.000 ASCs (n=9) or vehicle (n=14) intravenously. The effects of ASC treatment on the size and stability of aortic root atherosclerotic plaques were determined 4 weeks post-treatment via (immune)histochemical analyses. Moreover, plasma monocyte subsets within 3 days pre- and 3 days post-treatment, as well as 4 weeks post-treatment, were studied.

Results: ASC treatment did not significantly affect atherosclerotic plaque size or intra-plaque inflammation. ASC-treated mice had a higher percentage of intra-plaque fibrosis (42.5±3.3%) compared to vehicle-treated mice (37.6±6.8%, p=0.07), albeit without reaching statistical significance. Additionally, the percentage of inflammatory classical peripheral circulating monocytes was significantly increased by ASC therapy within 3 days post-treatment (p=0.005), but significantly decreased 4 weeks post-treatment (p=0.003) compared to pre-treatment.

Conclusions: Adipose-derived MSCs did not affect atherosclerotic plaque size or intra-plaque inflammation in a DM mouse model, when using a clinically relevant MSC dose. However, ASC therapy induced a borderline significant increase in intra-plaque fibrosis and a shift towards an anti-inflammatory phenotype in circulating monocytes 4 weeks post-treatment. These results thus indicate potential benefit of ASC therapy in DM-related atherosclerosis, and warrant further investigation.

Poster Abstract 55

Presenter: Jiuru Li

Heart Failure & Arrhythmias

Novel hiPSC-atrioventricular canal cardiomyocytes to unravel LMNA associated electrical dysfunction

Jiuru Li, Alexandra Wiesinger, Lianne Fokkert, Priscilla Bakker, Arie Verkerk, Dylan De Vries, Anke Tijsen, Yigal Pinto, Geert Boink, Vincent Christoffels, Harsha Devalla

Medical Biology Location AMC

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are an excellent tool for studying cardiac ontogenesis, and modeling human disease. Tremendous advances have been made in the last decade to develop protocols that yield specialized cardiomyocyte types such as sinoatrial nodal, atrial, and ventricular cells. Approaches to differentiate critical cardiomyocyte subtypes such as cells of the atrioventricular canal (AVC) or that of ventricular conduction system are currently lacking. In this study, we aimed to generate AVC-like cardiomyocytes from hiPSCs and investigate their ability to model cardiac conduction diseases in vitro.

Our results revealed that modulation of WNT and Retinoic acid (RA) signaling at the cardiac mesoderm stage yields a cell population that preferentially express MSX2, TBX2, and TBX3. Single cell RNA sequencing confirmed that these cells are most similar to AVC cluster of the mouse heart. To evaluate whether hiPSC-AVC cardiomyocytes could be used to model the atrium-ventricle conduction axis, we created a novel organoid-based tissue model. These “assembloids” consisting of atrial, AVC, and ventricular organoids demonstrated unidirectional conduction and characteristic “fast-slow-fast” conduction pattern found in the heart tube during embryogenesis.

We then tested the potential of hiPSC-AVCMs in modeling cardiac conduction disease caused by mutations in LMNA. Single cell patch-clamp revealed a higher presence of delayed after depolarizations (DADs) in LMNA mutant hiPSC-AVCMs compared to isogenic controls. In addition, assembloids, generated from LMNA hiPSCs-CMs exhibited a higher prevalence of conduction block when paced at 2Hz and 3Hz compared with isogenic control assembloids. Remarkably, treatment of assembloids with S107, a RYR2 stabilizing compound, was able to alleviate the block phenotype in 80% of treated assembloids. In summary, our results demonstrate that hiPSC-derived AVC cardiomyocytes combined with a novel assembloid model are valuable tools for modeling complex cardiac conduction disorders.

Poster Abstract 56

Presenter: Robin Lierop

Heart Failure & Arrhythmias

Allele-specific shRNAs to treat RBM20 cardiomyopathy

Robin M.J. van Lierop, Yigal M. Pinto, Anke J. Tijsen

Experimental Cardiology Location AMC

Dilated cardiomyopathy (DCM) is a progressive myocardial disease, characterized by enlargement of one or both ventricles. Around 3% of the familial DCM cases are caused by autosomal dominant mutations in the RNA-binding motif protein 20 (RBM20), which is a splicing factor involved in the regulation of several cardiac genes involved in for instance sarcomere assembly and calcium handling. RBM20 mutations cause a clinically aggressive phenotype, characterized by fast progression of heart failure, increased risk of arrhythmias and high mortality. Unfortunately, these patients do not respond to current evidence-based therapies for heart failure, which indicates the need to develop new therapeutics.

Recently, our group successfully used allele-specific short hairpin RNAs (shRNAs) to inhibit the mutant allele in LQT1 human-induced pluripotent stem-cell-derived cardiomyocytes (hiPSC-CMs), which improved their disease phenotype. We intend to investigate a similar approach as a potential treatment for patients with RBM20 mutations. However, testing of multiple allele-specific shRNAs in hiPSC-CMs is an expensive and time-consuming effort. Therefore, we aim to develop a novel screening method, which would enable us to more easily screen the efficacy and allele-specificity of all potential shRNAs.

To perform this screen, a dual reporter with firefly and renilla luciferase under control of a bidirectional promotor was constructed. We cloned a small part of the wild-type and mutant allele in the coding region of the firefly and renilla luciferase respectively. Co-transfection of this reporter with allele-specific shRNAs into HEK-cells will enable us to determine efficacy and allele-specificity of these shRNAs based on the firefly and renilla luciferase activity. This reporter system is currently being validated and optimized using our previously developed allele-specific shRNAs targeting the KCNQ1 gene. Initial experiments have shown changes in luciferase activity corresponding with the previous findings in hiPSC-CMs. The optimized parameters will later be used to investigate allele-specific shRNAs targeting the RBM20 gene. Subsequently, the best RBM20 shRNAs will be used in heterozygous RBM20 mutant hiPSC-CMs to determine their effects on the disease phenotype and explore their therapeutic potential.

Poster Abstract 57

Presenter: Alex Lipov

Heart Failure & Arrhythmias

Identification of likely deleterious rare non-coding variants in Brugada syndrome using whole-genome sequencing

Alex Lipov, Roddy Walsh, Connie Bezzina

Experimental Cardiology *Location AMC*

Background: Brugada syndrome (BrS) is an inherited cardiac condition characterised by ST-segment elevation in the right precordial ECG leads and an increased risk of sudden cardiac death. Approximately 20% of BrS cases have a rare pathogenic or likely pathogenic exonic variant in SCN5A. The genetic aetiology in the other 80% of cases is not known. The largest GWAS of BrS identified common non-coding risk variants across 12 different loci—however, the role of rare non-coding variants, which have larger effect sizes, remains unexplored.

Aim: Ascertain a portion of the missing heritability in BrS by identifying rare deleterious non-coding variants that disrupt enhancer sequences.

Methods: Whole genome sequencing (WGS) was performed on 424 BrS cases and 801 controls from the UK and Netherlands. Variants were examined that were within ± 0.5 Mb of a set of genes identified in a prior BrS GWAS, with randomly selected genes from the genome also included as controls. Variants were filtered to ultra-rare variants (gnomAD MAF in NFE < 5e-4) that were enriched in cases. These were further filtered to those given a score of ≥ 0.7 by FINSURF—a recently developed random-forest machine learning model that predicts non-coding variants with high pathogenic impact using an annotation set that includes conservation and epigenetic data.

Results: Rare non-coding variants of interest were identified, all within BrS GWAS loci. This included a variant in intron 19 of SCN5A predicted to disrupt a conserved ETS-family TF binding motif (3-38605250-C-G, GRCh37; 5 cases, 0 controls). Another two variants overlap known enhancers from the VISTA database—one in a gene desert upstream of TBX20 (7-35458992-G-A; 3 cases, 0 controls) and the other in an intergenic region between IRX3 and IRX5 (16-54576963-C-T; 3 cases, 0 controls).

Conclusion: WGS has enabled the first identifications of likely deleterious rare non-coding variants in BrS.

Poster Abstract 58

Presenter: Aida Llucià-Valldeperas

Pulmonary Hypertension & Thrombosis

Atrial and Brain Natriuretic Peptides Expression and Release in Pulmonary Hypertension

Aida Llucià-Valldeperas, Jessie van Wezenbeek, Rowan Smal, Fjodor Bekedam, Anton Vonk-Noordegraaf, Harm-Jan Bogaard, Frances de Man

Pulmonary Medicine *Location Vumc*

Introduction: Precapillary pulmonary hypertension (PH) is characterized by progressive overload for the right ventricle and, eventually, right heart failure (RHF). N-terminal Brain Natriuretic Peptides (NTproBNP) and Atrial Natriuretic Peptides (ANP) are secreted from cardiomyocytes upon stretch and are important biomarkers in Heart Failure.

Aim: To study the association between ANP and NTproBNP and pressure overload in blood, cardiac tissue, and induced pluripotent stem cell derived cardiomyocytes (iPSC-CMs) from PH-patients and healthy subjects.

Methods: We measured NPPA and NPPB gene expression on end-stage human right ventricular (RV) and right atrial (RA) samples from PH-patients (8 RV and 4 RA) and controls (5 RV and 8 RA). We analyzed ANP and NTproBNP release in 9 PH-patients before and 6-months after pulmonary endarterectomy (PEA). In addition, iPSC-CMs derived from 1 male and 1 female PH-patient and healthy control were stretched for 24h at 1Hz and 10% on the Flexcell FX-6000 system, ANP and BNP gene expressions, and NTproBNP release were measured on static and stretched iPSC-CMs. ANP and BNP gene expression were quantified via RT-PCR. ANP and NTproBNP protein levels were measured throughout ELISA and ECLIA, respectively.

Results: In RV tissue of PH-patients ANP gene expression was ~20-fold upregulated, while in RA tissue of PH-patients BNP gene expression was ~2-fold greater. Pressure unloading in CTEPH patients decreased ANP, but not NTproBNP levels. Furthermore, male and female iPS-CMs showed different levels of ANP and BNP production under static and stretched conditions. Finally, ~5x higher NTproBNP was released from male PH-derived iPSC-CMs under static conditions but also after mechanical stretch, compared to female PH-derived iPSC-CMs.

Conclusions: ANP and BNP showed important differences at gene and protein levels in relation to pressure overload and gender. To confirm current results, further analyses on atrial and ventricular iPSC-CMs will be performed, in addition to mid-range proANP measurements in patients' blood and iPSC-CMs conditioned medium.

Poster Abstract 59

Presenter: Eva Lukas

Heart Failure & Arrhythmias

Unraveling the Complex Interplay of PTSD and Cardiovascular Disease: Genomic Insights through MR and gSEM

Eva Lukas, Rada R Veeneman, Dirk JA Smit, Michel G Nivard, Jentien M Vermeulen, Gita A Pathak, Renato Polimanti, Karin JH Verweij, Jorien L Treur

Psychiatry Location AMC

Introduction: Observational studies have revealed a notable increase in cardiovascular disease (CVD) among individuals suffering from post-traumatic stress disorder (PTSD). Exploring potential causality and association between PTSD and CVD holds significant clinical relevance, as it paves the way for tailored interventions and proactive measures aimed at reducing the heightened cardiovascular risk in individuals with PTSD. In this study, our primary objective was to elucidate both the genetic basis and potential causality driving this association using Genome Wide Association Study summary data.

Methods: We employed advanced methodologies, including Genomic Structural Equation Modeling (gSEM), as well as uni- and multivariate Mendelian randomization (MR). Furthermore, we examined potential mediators from three distinct perspectives: behavioral, immunological, and neurological.

Results: Our approach defined genetic correlations and common genetic factors, substantiating our hypothesis of a shared genetic factor connecting cardiovascular traits and PTSD. Mediation models revealed that for coronary artery disease (CAD) and heart failure, behavioral traits predominantly mediated the relationship with PTSD. In other effects, both behavioral and immunological mediators played significant roles. Lifestyle traits emerged as dominant mediators in the regression of PTSD on the common genetic factor of cardiovascular disease, while the reverse relationship was characterized by the influence of both lifestyle and immunological markers. We also explored individual Single Nucleotide Polymorphism effects mediated by PTSD on indicators of the common genetic factor, highlighting the impact of PTSD on CAD. Using genetic instrumental variables within an MR framework, we identified potential causal links, particularly between PTSD and CAD.

Conclusion: These insights underscore the importance of adopting a holistic approach to address the complex physical and mental health challenges faced by individuals with PTSD. They provide a solid foundation for future research aimed at fully comprehending the intricate mechanisms that connect these conditions and developing effective interventions.

Poster Abstract 60

Presenter: Madelief Marsman

Heart Failure & Arrythmias

Consortin: a potential modulator of ventricular conduction

E. Madelief Marsman, MD; Joost A. Offerhaus, MD; Fernanda M. Bosada, PhD; Leander Beekman; Vincent M. Christoffels, PhD; Bas J. Boukens, PhD; Carol Ann Remme, MD, PhD; Connie R. Bezzina, PhD

Experimental Cardiology Location AMC

Background: Consortin, encoded by the CNST gene, is a ubiquitously expressed protein putatively involved in trafficking of connexins to the plasma membrane. In the heart, connexins form gap junctions, which are essential for cardiac conduction. Reduced levels and abnormal cellular distribution of connexins have been found in patients with heart failure, atrial fibrillation, arrhythmogenic cardiomyopathy and Brugada syndrome.

Purpose: To decipher the role of Consortin in cardiac electrophysiology.

Methods: Homozygous Cnst knockout mice (Cnst-/-) were generated using CRISPR-Cas9 technology. Consortin expression was assessed by Western blot analysis. Cardiac conduction parameters were recorded by electrocardiography in anesthetized wild-type (WT) and Cnst-/- male and female littermates aged 3-6 months. Conduction velocity (CV) was determined in Langendorff-perfused hearts by optical mapping of the right ventricle (RV) at a pacing cycle length of 120 ms. Data were analyzed by Student's t¬-test and P values < 0.05 were considered significant.

Results: Western blot analysis confirmed the presence of Consortin in WT mouse hearts, and its absence in Cnst-/- hearts. Heart rate, P-wave duration and PR-interval did not differ between groups, whereas QRS interval was significantly prolonged in Cnst-/- (mean±SEM 8.7±1.3 ms, n=13) compared to WT mice (7.9±1.1ms, n=13; (p=0.0009)). Hearts were structurally normal, and heart weight:body weight ratio was similar between groups. Preliminary optical mapping at the RV of Langendorff-perfused hearts demonstrated a lower, although not significantly different, transversal CV in Cnst-/- (56±4.3 cm/s, n=6) compared to WT (65±7.1 cm/s; n=3; p=0.31), whereas longitudinal CV was similar between groups (Cnst-/- 80±4.2 cm/s versus WT 83±9.1 cm/s; p=0.74). No differences were observed in RV effective refractory period (p=0.59).

Conclusion: These preliminary findings support a modulatory role of Consortin in ventricular conduction.

Current efforts are aimed at elucidating the underlying electrophysiological and molecular mechanisms, including the impact of Consortin on cardiac connexins.

Poster Abstract 61

Presenter: Madelief Marsman

Heart Failure & Arrythmias

Investigating the role of WT1 in Brugada syndrome pathophysiology

Marsman EM, Offerhaus JA, Bosada FM, Beekman L, Wilde LM, Casini S, Verkerk AO, Wilde AAM, Remme CA, Boukens BJ, Bezzina CR

Experimental Cardiology Location AMC

Brugada syndrome (BrS) is a heritable arrhythmic disorder presumably caused by a reduced conduction reserve in the right ventricular (RV) outflow tract (RVOT). A recent genome-wide association study found an association between a genetic locus in Chr11, near the transcription factor Wilms' Tumor (WT1), and BrS. However, the underlying mechanism(s) remain unknown. Here, we investigate the impact of this gene on cardiac conduction reserve and thereby BrS pathophysiology.

Chromatin profiles of putative regulatory elements (REs) located within the BrS-associated region associated with the expression profile of WT1. Human cardiac promoter capture Hi-C showed interactions between the promoter of WT1 and the BrS-associated region. Wt1+/- mice were employed to explore the potential impact on cardiac conduction. We did not detect any differences on in vivo electrocardiograms between Wt1+/- and littermates. Optical mapping demonstrated that epicardial conduction of the RV and RVOT was unaffected in Langendorff perfused mutants. Patch-clamp studies on enzymatically isolated RV cardiomyocytes showed that reduction of Wt1 does not affect action potential and sodium current characteristics. Transcriptomic analysis of RV outflow tract and RV cardiomyocytes revealed a significant but modest increase in Scn5a expression in Wt1+/- vs control littermates. This suggested that Wt1 reduction can be protective in the context of cardiac conduction. To investigate a potential functional impact of Wt1 in the setting of reduced conduction reserve, Wt1+/- mice were crossed with Scn5a+/- mice. While ageing exacerbated the reduced conduction reserve present in Scn5a+/- mice, this was independent of reduced Wt1 expression. Further reduction of the conduction reserve by acute administration of the sodium channel blocker ajmaline or by pacing at the effective refractory period revealed that reduced expression of Wt1 was associated with faster conduction in aged Scn5a+/- mice.

We identify WT1 as the most likely gene driving the previously identified association between the locus in Ch11 containing rs72905083 and BrS. Reducing Wt1 expression did not affect cardiac electrophysiological properties in mice under normal physiological circumstances. However, the setting of extreme reduced conduction reserve revealed protective effects of decreased expression of Wt1.

Poster Abstract 62

Presenter: Kevin Mol

Microcirculation

Mechanisms of micro-thrombus extravasation in the mouse brain: experimental model development

K. Mol, H.E. de Vries, E.T. van Bavel, I.A. Mulder

Biomedical Engineering and Physics Location AMC

Keeping the brain's microcirculation perfused is crucial to maintaining proper brain function. However, throughout life and with increased frequencies during aging, microvessels may become occluded, resulting in micro-strokes, also known as "silent brain infarcts", ultimately leading to possible brain damage and cognitive decline. Fortunately, the microvasculature may become reperfused in the hours and days after obstruction by respectively the fibrinolytic system or by the extravasation of the occluding particles through the blood-brain barrier (BBB), also known as angiophagy. However, angiophagy has not been extensively studied. In this project, we gain a better understanding of the mechanisms that enable the reperfusion of the microcirculation via angiophagy.

In a recently started project, we aim to identify the molecular mechanisms that are involved in angiophagy by using the micro embolism model to mimic the small vessel occlusions in mice. Combined with a chronic cranial window and in vivo multi-photon microscopy imaging we can follow angiophagy of microvascular occlusions over time. By using transgenic mice we can visualize and identify the role of endothelium and pericytes during angiophagy.

Additionally, We optimized an in vitro model using hCMEC/D3 cells to study the transmigration of polystyrene microparticles. Preliminary results show that cells form endothelial pores that enable polystyrene microparticles to transmigrate through the monolayer. Shortly, we will start with the first pilot experiments to follow angiophagy in a vessel-on-a-chip model using iPSCs.

To conclude, this project will improve our understanding of how angiophagy enables the reperfusion of the microvasculature and potentially contribute to the development of new treatment strategies for silent brain infarcts.

Poster Abstract 63

Presenter: Ali Nassar

Heart Failure & Arrhythmias

Human cultured cardiac tissue slices to study disease mechanisms and test drugs in hypertrophic cardiomyopathy

Ali Nassar, Vincent Warnaar, Michelle Michels. Andrease Dendorfer, Diederik Kuster, Jolanda van der Velden.

Physiology Location Vumc

Hypertrophic cardiomyopathy (HCM) is the most prevalent genetic cardiomyopathy. It is characterized by left ventricular hypertrophy, preserved or elevated systolic function and diastolic dysfunction. The genetic basis of HCM is diverse, moreover in half of HCM patients no pathogenic gene variant is identified. Pathomechanisms differ depending on the presence of a mutation and the affected gene, leading to diverse drug responsiveness. There is a necessity for human models that recapitulate cardiac physiology and capture variability between patients.

We use the MyoDish platform (InVitroSys) for long-term culture of cardiac tissue slices from HCM patients that underwent septal myectomy surgery. Tissue is cut in 300µm thick slices using a vibratome and inserted into a biomimetic culture chamber. Tissue is stretched to achieve preload, electrically stimulated at 0.5 Hz at 37ºC and force of contraction is constantly recorded. We hypothesize that the viability, structural integrity, transcriptome and metabolome is maintained after long-term culture. Slices were prepared from 8 HCM patients. At least 4 slices were successfully cultured for 28 days from each patient tissue. To test the effect of long-term culture we compare slices from different time-points (Day 14, and 28) to slices at day 0. Confirming previous studies, we observe a relatively large variation in contractile function between patients. We show that tissue slices from HCM patients represent a useful system to capture patient-to-patient variability. This method allows for more accurate disease characterization and long term drug testing with a patient specific approach.

Poster Abstract 64

Presenter: Soufiane Nassiri

Heart Failure & Arrhythmias

GeranylGeranylAcetone for Heart failure with preserved ejection fraction (GLADIATOR-HFpEF)

Soufiane Nassiri, Geert Voordes, Adriaan Voors, Loek van Heerebeek, Daniël van Raalte, Ed Eringa, Louis Handoko**

Physiology Location Vumc

Background: Geranylgeranyl-acetone (GGA) was proven to ameliorate cardiomyocyte stiffness in a ZSF-1 rat model of heart failure with preserved ejection fraction (HFpEF) by increasing myofilament binding of heat shock proteins αB-crystallin and HSP27. Furthermore, in healthy subjects, a single oral dose of GGA enhances HSP90 expression and peripheral endothelial function, which independently relates to risk of HFpEF1. In the GLADIATOR-trial, we hypothesize that administering GGA ameliorates diastolic function and improves endothelial function in patients with HFpEF.

Aim: To evaluate GGA for treatment of heart failure with a preserved ejection fraction, and the roles of improved microvascular function and reduced cardiomyocyte stiffness.

Methods/design: GLADIATOR-HFpEF is a phase 2 multi-centre randomized double-blind place-controlled cross-over trial comparing the efficacy of GGA-treatment in 40 patients ≥ 50 years with diagnosed HFpEF. Patients receive 300 mg oral GGA treatment once daily and matching placebo in a crossover model for 3 months.

Study endpoints: Co-primary endpoints are myocardial stiffness measured by transthoracic echocardiography (TTE; E/e') and peripheral microvascular function measured as reactive hyperemia by arterial tonometry (EndoPAT). Secondary endpoints include systolic and diastolic myocardial function, peripheral endothelial and smooth muscle function measured by laser speckle contrast analysis, invasive renal function measurements, and HFpEF symptoms measured as the six minute walking distance and the Kansas City Cardiomyopathy Questionnaire. Potential benefit for these patients might be improved LV diastolic function.

Expected effect: we expect that 3 months of GGA treatment has a favourable effect on diastolic function or endothelial dysfunction, the main drivers for HFpEF, providing a basis for further clinical development.

1. Canto ED et al. Cardiovascular Diabetology. 2023;22:234.

Poster Abstract 65

Presenter: Molly O'Reilly

Heart Failure & Arrhythmias

Exploring the role of neuronal dysfunction in inherited arrhythmia syndromes

Molly O'Reilly, Fernanda Bosada-Musselwhite, Tanja de Waal, Marieke Veldkamp, Simona Casini, Carol Ann Remme

Experimental Cardiology *Location AMC*

Background: Inherited arrhythmia syndromes are a leading cause of sudden cardiac death in young and otherwise healthy patients. These are often caused by mutations in genes that encode ion channels or transporters, leading to conditions such as Brugada Syndrome (BrS), Long QT Type 3 (LQT3), and Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT).

BrS and LQT3 are associated with mutations in SCN5A (encoding sodium channel, Nav1.5), whilst CPVT is caused by mutations in RYR2 (encoding ryanodine receptor 2, RyR2). Investigation into the effects of these mutations have exclusively been performed in cardiac cells. However, these ion channels are also present in neuronal tissue, including intracardiac neurons (which modulate cardiac function). Moreover, patients often present with clinical signs of dysfunction of the autonomic nervous system.

Aim: To investigate the neuronal phenotype induced by these ion channel mutations, using arrhythmia mouse models to assess functional alterations as well as broader autonomic remodelling. Ultimately, we will assess the contribution of this to arrhythmogenesis.

Results: Using immunohistochemistry approaches, we reveal for the first time that both Nav1.5 and RyR2 are expressed in mouse stellate ganglia (a crucial autonomic modulator of cardiac function). Current functional investigations, including patch clamp analysis, are aimed at assessing the functional consequences of Scn5a and Ryr2 mutations in stellate and intracardiac ganglia in mouse models of BrS, LQT3 and CPVT. In addition, autonomic remodelling in stellate/intracardiac ganglia and (ventricular) myocardium is currently being assessed through RNA sequencing, immunohistochemistry in mouse heart cryosections, and whole-heart neuronal imaging. Findings from these investigations will be presented.

Conclusions: All neurons that modulate heart function (stellate ganglion and intracardiac neurons) express Nav1.5 and RYR2. Mouse models of BrS/LQTS/CPVT are employed to investigate the consequences of Scn5a/RyR2 mutations on neuronal (dys)function and their potential role in arrhythmogenesis.

Poster Abstract 66

Presenter: Adriana Passadouro

Diabetes & Metabolism

Metabolomics and lipidomics insights into Barth syndrome cardiomyopathy

Adriana S. Passadouro (1,2,3), Bauke V. Schomakers (4), Michel van Weeghel (4), Denise Cloutier (5), Barry J. Byrne (6), Jan Bert van Klinken (1,3,4,7), Paul M. L. Janssen (8), Frédéric M. Vaz (1,3,4,9), Jolanda van der Velden (2,10), Riekelt H. Houtkooper (1,2,3,9) and Signe Mosegaard (1,2,3,9)

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Genetic Metabolic Diseases *Location Vumc*

Barth syndrome (BTHS) is a rare X-linked recessively inherited disorder caused by variants in the TAFAZZIN gene. The pathogenic variants lead to impaired conversion of monolysocardiolipin (MLCL) into matured phospholipid cardiolipin (CL). The accumulation of MLCL and mature CL deficiency is a diagnostic marker for BTHS. The clinical spectrum includes cardiomyopathies, skeletal myopathies, neutropenia, and delays in growth and development. In severe BTHS patients, the cardiac phenotype is early onset, heterogeneous and unpredictable. Ultimately, severely affected patients require a cardiac transplantation early in their life. Unfortunately, the pathophysiological mechanisms of BTHS are poorly understood, and treatment options for BTHS remain symptomatic.

In this study, we investigated a unique collection of heart samples from five paediatric male BTHS patients (5 month-15 years old) and 24 non-failing donors (19-71 years old). We performed metabolomics and lipidomics using UPLC-mass spectrometry (LC-MS). The lipidomic profile of BTHS confirmed the findings of MLCL accumulation and CL depletion, corroborating what has been reported in literature. In addition, the acylcarnitine profile showed significantly decreased long-chain acylcarnitines in the BTHS samples, suggesting a possible shift in energy metabolism from fatty acid oxidation to glycolysis. Metabolomics showed significantly decreased metabolites including adenosine triphosphate (ATP), creatine phosphate, acetyl-CoA and carnitines (short, medium and long-chain), indicative for energy deficiency. Increased metabolites included pyruvate, lactate, alanine and serine, which point to a metabolic preference towards glycolysis.

Our analysis comparing heart biopsies from BTHS individuals to non-failing donors, reveal that BTHS have a unique and distinct metabolic and lipidomic profile. Our findings suggest that the BTHS heart can undergo a metabolic switch from fatty acid oxidation to glycolysis when compared to control. The latter switch may represent a compensatory mechanism in response to cardiac energy deficiency.

Poster Abstract 67

Presenter: Merel Peletier

Microcirculation

Investigating the inflammatory - metabolic axis in valve interstitial cells as target for reducing the progression of aortic valve stenosis

Merel Peletier1,2, Lubna Ali1,2, Tarik el Bouazzati1,2, María Leonor Romero Prats1,2, Kim Dzobo1,2, Miranda Versloot1,2, Jorge Peter1,2, Sotirios Tsimikas3, Mark Dweck 4 and Jeffrey Kroon1,2,5,6

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Experimental Vascular Medicine Location AMC

Aortic valve stenosis (AVS) is a disease affecting the aging population characterized by inflammation-induced cellular changes and extensive valve calcification. Recently, lipoprotein (a) [Lp(a)] has been considered to play a crucial role in AVS pathophysiology. Despite open heart surgical valve replacement is the conventional treatment, there is an urgent demand for low-risk, non-invasive pharmaceutical interventions. Valve Interstitial Cells (VICs) play a crucial role in the progression of AVS. Notably, AVS and atherosclerosis exhibit shared characteristics, prompting our hypothesis that Lp(a) can modulate the metabolic-inflammatory axis in VICs, ultimately causing calcification.

Using 18F-FDG PET/CT, we found increased inflammatory activity (TBRmax 1.6 vs. 1.4) in heart valves of high Lp(a) (> 50 mg/dl) vs. low Lp(a) (< 50mg/dl) patients, indicating elevated glucose uptake. In vitro Lp(a)-stimulated VICs a(100 mg/dl) exhibited a 40-fold IL-8, 2-fold IL-6 and 7-fold MCP-1 increase. This coincided with a simultaneous increased glucose consumption and lactate secretion (1.25-fold). Radioactive tracer experiments using tritium-labeled glucose corroborated these findings of increased glycolysis.

Inhibiting the key-glycolytic enzyme PFKFB3, using KAN0438757 reduced glucose uptake, glycolytic activity, as well as the maximal glycolytic capacity. This aligned with a 50% reduction in cytokine secretion (IL-6 and MCP-1), emphasizing the intricate link between PFKFB3-driven glycolysis and inflammation in VICs. These findings highlight metabolic reprogramming as a crucial factor in the inflammatory status of VICs during AVS initiation.

In order to investigate the effects of PFKFB3-driven glycolytic inhibition in VICs on immune cell (PBMC) influx, we utilized an endothelial/VICs co-culture model. Lp(a) stimulation of VICs (without modulation of the endothelial component), led to a profound increase in PBMC influx in the subendothelial compartment, which could be reduced after glycolytic inhibition. This model shows the role of VIC metabolic modulation in reducing the inflammatory microenvironment of the heart valve. Collectively, this research provides insights into potential therapeutic strategies for targeting metabolic mechanisms underlying Lp(a)-induced AVS."

Poster Abstract 68

Presenter: Philippa Phelp

Microcirculation

Human pulmonary microvascular endothelial cell barrier response to plasma of patients with hypo- and hyperinflammatory acute respiratory distress syndrome subphenotypes

P. Phelp, L. Atmowihardjo*, R. van Amstel, A. Tuip-de Boer, R. Ibelings, L. Bos, C. van den Brom*

Department of Intensive Care Medicine Location AMC

Background and aim: Alveolar-capillary hyperpermeability is one of the hallmarks of Acute Respiratory Distress Syndrome (ARDS). Two ARDS subphenotypes have been identified, differing in biological characteristics such as inflammation and endothelial dysfunction. To study the relationship between endothelial permeability and ARDS heterogeneity, this study aimed to examine the effect of ARDS patient plasma with different subphenotypes on pulmonary microvascular endothelial cell (PMVEC) permeability.

Methods: Forty patients with ARDS were selected based on a hyper- or hypoinflammatory subphenotype (n=20 per classification) from a previously performed prospective observational cohort study. Confluent PMVECs were exposed to 10% citrated plasma for 6 hours and in vitro endothelial barrier function was assessed using electric cell-substrate impedance sensing. The effect of plasma administration on endothelial cell (EC) barrier function was analyzed and clinical and plasma biomarker data was stratified by response.

Results: Patients of the hyperinflammatory subphenotype were characterized by more severe organ failure and higher ventilator requirements. There was no difference in median resistance between hypo- versus hyperinflammatory plasma (0.64[0.6-0.76]Ω vs. 0.67[0.59-1.1]Ω, p=0.59). Dichotomization to above vs. below 0.7 normalized resistance resulted in a group with preserved barrier function (n=24) and a group with increased permeability (n=56) with median normalized resistance of 1.22[1.08-1.33]Ω vs. 0.61[0.58-0.65]Ω (p<0.001). The latter had a lower median SOFA score (9[7-11] vs. 13[11-15], p<0.001) and more favorable measures of organ failure, such as lower lactate levels (3.3[1.7-6.2]mmol/L vs. 8.5[3.8-11.1]mmol/L, p=0.002).

Conclusion: ARDS patient plasma induced a heterogeneous response on in vitro endothelial barrier function, with no difference in endothelial permeability between hypo- and hyperinflammatory ARDS subphenotypes. Unexpectedly, plasma of patients with less severe organ failure caused more severe endothelial barrier disruption, the reason for which is presently unknown.

Poster Abstract 69

Presenter: Fabienne Podieh

Microcirculation

Proteomics screen to identify new regulators of endothelial barrier function

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Physiology *Location Vumc*

Endothelial cells (ECs) form a semi-permeable, dynamic barrier controlling the passage of plasma and leukocytes from the circulation into the tissue. By controlling EC cytoskeletal dynamics and stability of junctions, Rho GTPases are key modulators of endothelial integrity. Signaling by Rho GTPases is regulated in several ways, including ubiquitination-mediated degradation, which requires the sequential activity of E1, E2 and E3 ligases. Previously, it was shown that ubiquitination and degradation of the Rho GTPase RhoB is crucial to preserve quiescent endothelial barrier function. Our recent study shows that ubiquitination has a more generic role in regulating endothelial barrier function and that there are other barrier regulators, next to RhoB, which have a short half-life and are controlled by continuous ubiquitination and degradation.

To identify such proteins and their role in endothelial integrity, we performed short-term inhibition of E1 ligase and Cullin-mediated E3 ligases in primary human ECs and identified up- and downregulated proteins in a proteomics approach. After data analysis and literature search, knockdown of the six most promising hits was performed and the effect on endothelial integrity assessed. Silencing of angio-associated migratory cell protein (AAMP) results in marked improvement of endothelial barrier function. Concomitantly, immunofluorescent staining shows an increase in junctional, VE-cadherin-positive area and in total cell area, indicating increased cell spreading in ECs following loss of AAMP expression. We are currently investigating the mechanism by which AAMP acts on endothelial barrier function, will identify downstream targets of AAMP and analyze if AAMP itself is regulated by ubiquitin-mediated degradation in ECs. Thus, we identified AAMP as novel negative regulator of endothelial integrity. This is important, as knowledge on the regulation of endothelial integrity will contribute to our options to target dysregulation of vascular permeability.

Poster Abstract 70

Presenter: Koen Prange

Atherosclerosis & Ischemic Syndromes

Human atherosclerotic plaque macrophages associate with clinical presentation and follow diverse lineage differentiation routes

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MBIOC Location AMC

Atherosclerosis is characterized by inflammation and lipid accumulation, leading to complications such as stroke and myocardial infarction. Various macrophage subsets are present in human atherosclerotic plaques. However, it is unclear how subsets are interrelated and contribute to the disease process and its clinical outcomes.

Here, we employed single-cell RNA-seq (scRNA-seq) on blood and plaque material from carotid endarterectomy patients enrolled in the AtheroExpress (AE) cohort to define regulatory pathways of macrophage recruitment, differentiation, and development. Additionally, we used these scRNA-seq data to deconvolute a large AE bulk RNA-seq cohort to allow correlating cellular subsets in plaques with clinical traits.

Interestingly, we found that macrophages and their subsets are the only cell population significantly associated with major adverse cardiac events (MACE) during a 3-year follow-up period. Subsequently, we could divide plaque macrophages into 3 populations: inflammatory, tissue-resident / lipid-associated (TREM2 high), and foamy macrophages (TREM1 high). While the latter two are correlated with MACE, the inflammatory macrophages are inversely correlated with statin use.

Cellular fate analyses revealed two major paths of macrophage development in the plaque. First, non-classical monocytes entering the plaque, turning into inflammatory cells before becoming foamy and eventually dying. Second, resident macrophages that turn into either inflammatory- or lipid-associated macrophages, before meeting their foamy demise.

Together, these findings provide important insights on macrophages in atherosclerosis and suggest new leads to discover therapeutic targets for this disease.

Poster Abstract 71

Presenter: Mick Renkens

Atherosclerosis & Ischemic Syndromes

Characterizing pathophysiological patterns of coronary artery disease among Individuals with Elevated Lipoprotein(a)

Mick P.L. Renkens, MD1,2, Tsung-Yin Tsai MD2, Shigetaka Kageyama, MD2,, Pruthvi C Revaiah MD, MD2, Johan H.C. Reiber, PhD3, Robbert J. de Winter, MD, PhD1, Eduard Benit, MD, PhD4, Liesbeth Rosseel, MD, PhD5, Faisal Sharif MD, PhD2,6, Joanna J. Wykrzykowska, MD, PhD7,Yoshinobu Onuma MD, PhD2,6, Patrick W. Serruys MD, PhD2

Cardiology Location AMC

Background: Elevated serum levels of lipoprotein a (Lp(a), >50mg/dL) affect 20% of the worldwide population. These individuals are at high residual risk for ASCVD, driven primarily by coronary artery disease (CAD) and myocardial infarction. Despite its prevalence, little is known about the specific characteristics and patterns of CAD in these patients.

Methods: This is a prospective analysis in consecutive patients with elevated Lp(a) enrolled in the ongoing PIONEER IV trial, randomizing patients eligible for PCI between Quantitative Flow Ratio (Medis QFR, abnormal threshold<80) guided strategy vs usual care. In 3 major epicardial vessels, 3D-QCA analyses with Pressure Pullback Gradient (PPG, abnormal threshold <0.78) and instantaneous change in the pressure gradient (dQFR/ds, abnormal threshold >0.025) were done. The Virtual Pullback Curve, representing mean values, is reconstructed using .xml output (MedisSuite software). Chi-square test and one-way ANOVA were used for statistical comparisons with $p<0.05$ considered significant.

Results: Lp(a) levels were elevated in 21.6% of 568 patients. 3D-QCA analyses in 65 consecutive patients, evaluating 142 vessels revealed significant disease in 13/40 (32.5%), 25/52(48%), and 12/50(24%) in respectively RCA, LAD, and LCX ($p=0.3$) showed significant disease. Figure 1 summarizes the findings, by mean Virtual PPG curves, without significant difference among the 3 vessels ($p=0.72$ and $p=0.26$, respectively).

Conclusions: In patients with elevated Lp(a), flow-limiting CAD is associated with predominantly diffuse disease with a major gradient in all three epicardial vessels.

Poster Abstract 72

Presenter: Lotte Rijken

Microcirculation

Developing Trustworthy Artificial Intelligence (AI)-driven Tools to Predict Abdominal Aortic Aneurysm Progression and the Risk of Adverse Cardiovascular Events: the VASCULAID-RETRO Study

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Vascular Surgery *Location Vumc*

Preference for Poster presentation; I just started my PhD so we don't have any results yet and not a lot of material to present.

Introduction: To date, it is unknown which patients with an abdominal aortic aneurysm (AAA) will suffer cardiovascular events or in which patients the AAA will progress. The VASCULAID-RETRO study aims to develop artificial intelligence (AI) algorithms able to evaluate the extent of AAA disease progression and risk of cardiovascular events.

Methods: The VASCULAID-RETRO study aims to leverage retrospectively collected data of at least 5000 AAA patients from multiple European clinical centers for the development of AI-algorithms. Initially, a robust data infrastructure network will be established to gather standardized data from all six participating clinical centers. After collection of imaging, -omics data, and (reported) clinical patient data, AI-tools will be developed using this data. Automatic anatomical segmentation on images and image analysis on US, CTA and MRI will be performed. Moreover, prediction algorithms for each data type (imaging, -omics, and clinical data) will be created separately. These prediction algorithms will be merged using fusion AI models to build a comprehensive prediction algorithm based on multi-source data to generate overall risk scores or probabilities for AAA progression and the risk of cardiovascular events.

Results: Ethical approval for retrospective patient data collection have been secured by all clinical partners. Currently, the data infrastructure for the collection of the retrospective data is being developed. Patient data from electronic patient files will be collected in Castor EDC and imaging will be stored on an XNAT server.

Future perspective: The VASCULAID-RETRO AAA study is part of the VASCULAID project, an European Horizon-funded research project. Similar AI algorithms will be developed for patients with peripheral arterial disease (PAD) of the lower limbs. Following the VASCULAID-RETRO studies for AAA and PAD patients, prospective studies will be performed in which more data will be collected and the developed AI-algorithms will be validated for identifying AAA and PAD patients at high risk of disease progression and cardiovascular events.

Poster Abstract 73

Presenter: Karlijn Rombouts

Microcirculation

NUAK1 kinase activity regulates contraction in smooth muscle cells derived from abdominal aortic aneurysm patients

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Vascular Surgery & Physiology Location Vumc

Introduction: Abdominal aortic aneurysms (AAA) are defined as a weakening and dilatation of the aortic wall. The aim of this study is to investigate the underlying mechanism of altered in vitro contractility of vascular smooth muscle cells (SMC) derived from AAA patients, compared to control SMC (C-SMC).

Methods: Contractility of AAA-SMC (n=39) and C-SMC (n=18) was measured upon ionomycin stimulation using Electric Cell-substrate Impedance Sensor. A (phospho)proteomics analysis was performed in AAA-SMC (n=24) and control SMC (n=8). Integrative Inferred Kinase Activity (INKA) analysis was used to calculate kinase activity scores, based on phosphorylation data of either kinases or their substrates.

Results: AAA-SMC were divided into subgroups based on contraction (AAA-Low contracting: mean: 70,63%, SD: 5,51% (n=8); AAA-Normal contracting: mean: 83.69%, SD: 3.35% (n=22); AAA-High contracting: mean: 91.72%, SD: 1.22% (n=9)). Protein expression levels of Thrombospondin-1 ($r=.21$, $p= 0.0091$), PDZ and LIM domain protein 4 ($r=.46$, $p<0.0001$) and ATPase plasma membrane Ca2+ transporting 1 ($r=.17$, $p=0.018$) were correlated to SMC contraction, but siRNA mediated knock down of these proteins did not affect SMC contraction. INKA analysis identified NUAK1 kinase activity as potential regulator of SMC contraction, by phosphorylation on 3 amino acids on myosin phosphatase (MYPT1). This was confirmed by a correlation between NUAK1 activity and phosphorylation levels of 2 MYPT1 phosphosites (Ser445 ($r=.24$, $p=0.0056$) and Ser910 ($r=.52$, $p<0.0001$)). Moreover, NUAK1 protein and RNA expression levels were correlated to SMC contraction and NUAK1 knock down decreased contraction in AAA-SMC, but not in C-SMC.

Conclusion: Impaired contraction of AAA-SMC is seen compared to C-SMC, and this is regulated by NUAK1 kinase activity and expression. Currently we are performing further experiments to explore how NUAK1 exactly regulates contraction in AAA-SMC. Finding proteins involved in AAA-SMC dysfunction can contribute to novel non-invasive treatment options for prevention and/or stabilization of AAA.

Poster Abstract 74

Presenter: Amand Floriaan Schmidt

Heart Failure & Arrhythmias

DCM-PROGRESS: predicting end-stage heart failure in non-ischemic dilated cardiomyopathy patients

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Cardiology Location AMC

Aims: Patients with non-ischemic dilated cardiomyopathy (DCM) are at considerable risk for end-stage heart failure (HF), requiring close monitoring to identify early signs of disease. We aimed to develop a model to predict the 5-years risk of end-stage HF, allowing for tailored patient monitoring and management.

Methods and results: Derivation data were available from a Dutch cohort of 293 DCM patients, with external validation available from a Czech Republic cohort of 235 DCM patients. Candidate predictors spanned patient and family histories, ECG and echocardiogram measurements, and biochemistry. End-stage HF was defined as a composite of death, heart transplantation, or implantation of a ventricular assist device. Lasso and sigmoid kernel support vector machine (SVM) algorithms were trained using cross-validation. During follow-up 65 (22%) of Dutch DCM patients developed end-stage HF, with 27 (11%) cases in the Czech cohort. Out of the two considered models, the lasso model (retaining NYHA class, heart rate, systolic blood pressure, height, R-axis, and TAPSE as predictors) reached the highest discriminative performance (testing c-statistic of 0.85, 95%CI 0.58; 0.94), which was confirmed in the external validation cohort (c-statistic of 0.75, 95%CI 0.61; 0.82), compared to a c-statistic of 0.69 for the MAGGIC score. Both the MAGGIC score and the DCM-PROGRESS model slightly over-estimated the true risk, but were otherwise appropriately calibrated.

Conclusion: We developed a highly discriminative risk-prediction model for end-stage HF in DCM patients. The model was validated in two countries, suggesting the model can meaningfully improve clinical decision-making.

Poster Abstract 75

Presenter: Amber Schonk

Microcirculation

Unraveling the role of long non-coding RNA KCNQ1OT1 in heart failure with preserved ejection fraction

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Physiology Location Vumc

Background: Life expectancy keeps rising with a high percentage of the population being over 65 years old. Heart failure with preserved ejection fraction (HFpEF) is strongly associated with aging and constitutes more than 50% of heart failure incidence. HFpEF is characterized by impaired cardiac relaxation and increased ventricular stiffness. Along with coronary microvascular endothelial dysfunction, myocardial fibrosis due to increased collagen deposition in the extracellular matrix is associated with diastolic dysfunction. Long non-coding RNAs (lncRNA), >200 nucleotide-long non-coding transcripts, have been found to play a role in cardiovascular disease. However, their role in HFpEF is not known. Our RNA sequencing data from human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) cultured on soft and stiff matrices revealed lncRNAs, KCNQ1OT1 being highly upregulated in the stiff matrix environment, suggesting that it may be involved in stiffness-driven pathogenesis of HFpEF. We hypothesize that KCNQ1OT1 has detrimental effects on cardiac function. Inhibiting KCNQ1OT1 will improve diastolic function and cell viability. Our main goal is to study the role of KCNQ1OT1 in regulating cardiac function and dissect the underlying molecular mechanisms.

Methods: We will study the effects of KCNQ1OT1 through siRNA-mediated knockdown in hiPSC-CMs and examine the relaxation and contraction kinetics of the cells using a Cytocypher multicell analysis system. Furthermore, we will assess hiPSC-CM viability, apoptosis and proliferative capacity and identify protein binding partners of KCNQ1OT1 using mass spectrometry analysis to further decipher the downstream molecular pathway.

Results and Outlook: Our preliminary data demonstrates that KCNQ1OT1 knockdown improved CM relaxation (1.5 fold, $p<0.05$) and contraction kinetics (1.8 fold, $p<0.05$). KCNQ1OT1 silencing appears to increase cell confluency, suggesting its pro-proliferative or anti-apoptotic effect, which we will further investigate in this study. Our study will provide new insights on the contribution of lncRNAs, in particular KCNQ1OT1, to HFpEF pathogenesis establish the foundation for developing future therapies to improve cardiac function in patients with KCNQ1OT1 as a therapeutic target.

Poster Abstract 76

Presenter: Rianne Mirjam Schoon

Microcirculation

Novel binding protein KEAP1 directly regulates VE-cadherin to modulate endothelial barrier

Rianne Schoon1, Tsveta Malinova1, Floris van Alphen2, Maartje van den Biggelaar2, Jaap van Buul1,2, Stephan Huveneers1

Medical Biochemistry *Location AMC*

The endothelial cells lining the vessel wall maintain a selective barrier between blood and tissue. Structural connections between these cells are formed by adherens junctions where vascular endothelial (VE)-cadherin homophylic transmembrane adhesion protein connects the junctions to the actin cytoskeleton via a protein complex including α-, ß- and p120-catenin. We further explored the protein binding events to VE-cadherin that might dynamically regulate the barrier. By using mass-spectrometry following VE-cadherin pull-downs from endothelial cells we have identified novel proteins that interact independently of phosphorylation events at VE-cadherin's intracellular tail. After verifying their interaction using biochemical and co-localization techniques, we show that novel VE-cadherin binding protein KEAP1 functionally regulates adherens junctions. Following shRNA-based knock-down we find that loss of KEAP1 decreases VE-cadherin protein levels, resulting in reduced endothelial barrier function. However, our data demonstrates that KEAP1 is crucial for VE-cadherin trafficking but even though KEAP1 is associated with E3 ubiquitin ligase Cullin-3 it does not entail VE-cadherin ubiquitination. Furthermore, our data reveals that binding of KEAP1 to VE-cadherin is oxidant dependent, in line with KEAP1's function as an oxidative stress sensor affecting Nrf2 signaling. Together, our results indicate that the barrier is not exclusively controlled at cell-cell contacts, presenting KEAP1 as a direct regulator of VE-cadherin with a promising function in modulating vascular integrity.

Poster Abstract 77

Presenter: Ricky Siebeler

Atherosclerosis & Ischemic Syndromes

The histone demethylase KDM3A modulates macrophage pro-inflammatory phenotype in atherosclerosis

Ricky Siebeler, Marten A. Hoeksema, Hung-Jen Chen, Tanya Kuznetsova, Annette E. Neele, Saskia van der Velden, Marion J.J. Gijbels, Guillermo R. Griffith, Cindy P.A.A. van Roomen, Koen Prange, Menno P.J. de Winther

Medical Biochemistry *Location AMC*

The pervasive role of the innate immune system is regulated by interferons, in part by driving the inflammatory phenotype of macrophages. The transcriptional program of macrophages is tightly regulated by epigenetic enzyme-mediated histone modifications. In particular, the methylation status of histone H3 lysine 9 (H3K9), an epigenetic modification resulting in heterochromatin and gene repression, is important for inflammatory gene expression in macrophages. Here we identify KDM3A, the only demethylase for H3K9 to be upregulated during mouse atherosclerosis progression. Loss of KDM3A results in a hypo-inflammatory phenotype in both murine and human macrophages. We utilized computational approaches to reveal that interferon hypo-responsiveness underlies the phenotype of KDM3A deficient macrophages. By assessing genome-wide chromatin accessibility, KDM3A was found to regulate the accessibility of interferon regulatory factor motifs, thereby controlling the IFN response. As attenuated IFN signaling is likely to affect inflammatory disorders like atherosclerosis, we assessed the effects of KDM3A deletion in a murine disease model for atherosclerosis. Indeed, we found KDM3A deletion to impede the development of atherosclerotic lesions, prevent necrotic core formation, and reduce neutrophil recruitment. Overall, we conclude that KDM3A is a crucial regulator of IFN-driven inflammatory responses in macrophages and is therefore a compelling target in inflammatory disease.

Poster Abstract 78

Presenter: Nina Smets

Microcirculation

The role of perivascular spaces in brain clearance, assessed using two-photon microscopy

Nina G. Smets, Shakira van der Panne, Gustav J. Strijkers, Erik N.T.P. Bakker

Biomedical Engineering & Physics Location AMC

Brain clearance is crucial to brain homeostasis, and has gained much attention lately due to its possible involvement in the accumulation of proteins associated with neurodegenerative diseases like Alzheimer's disease, cerebral amyloid angiopathy, and Parkinson's disease. Perivascular spaces, together with the subarachnoid space, are considered a possible exit route for waste products from the brain into the lymphatic system. While the precise mechanism behind this clearance process remains elusive, numerous theoretical frameworks have been proposed. In the present study, we combined an acute cranial window with the administration of fluorescent dye injections in both the bloodstream and cerebrospinal fluid of mice. We subsequently analyzed the characteristics of the perivascular spaces using two-photon microscopy and ex vivo immunohistochemistry. The data reveal that sizes of perivascular spaces are irregular along pial arteries. Tracer distribution is confined to the perivascular spaces, with no infiltration into the arterial wall. These spaces are more abundant and larger around pial arteries compared to veins. Additionally, when encountering a junction involving both an artery and vein, some tracer dispersion is observed from the arterial perivascular spaces towards the venous regions, suggesting an interconnection of perivascular spaces between arteries and veins. Overall, these findings outline a network of perivascular spaces around arteries with limited involvement of veins. These results suggest that the removal of waste products from the brain is dominated by arterial perivascular spaces.

Poster Abstract 79

Presenter: Wilhelm Stehling

Microcirculation

A dynamic contrast-enhanced (DCE) MRI protocol optimized for scanning abdominal aortic aneurysms

Wilhelm Stehling, Myrte Wennen, Eva Aalbregt, Nienke Wassenaar, Eric Schrauben, Pim van Ooij, Kak Khee Yeung, Aart Nederveen, and Oliver Gurney-Champion

Radiologie Location AMC

Introduction: An abdominal aortic aneurysm (AAA) is a pathological dilation of the aortic wall. Rupture of an AAA results in high mortality rates. In current practice AAA diameter is utilized to assess the progression and risk of rupture, which is not precise enough to prevent rupture. Instead, measuring vessel wall microstructural perfusion may provide more information about vessel health and disease progression. DCE MRI promises to measure exactly this. However, current DCE protocols cannot accurately depict the vessel wall. Therefore we developed a protocol optimized for AAA patients to scan those with DCE MRI.

Methods: Typically, the aortic wall is hard to depict due to the bright signal arising from the blood in the aorta and fatty tissue surrounding the AAA. These bright signals are further disrupted and shifted by motion resulting in artifacts obscuring the signal of interest. To improve aortic wall visibility, we added a preparation pulse to our MRI sequence which removes the blood signal. Moreover, we added a fat suppression prepulse to suppress the signal of the surrounding fat. Due to these pulses the conventional DCE signal equation becomes insufficient. To overcome this, we developed new signal equations to enable pharmacokinetic modeling of DCE.

Furthermore, we used different motion compensation techniques in reconstruction and an advanced reconstruction method called compressed sensing. This enables us to scan patients with 1x1x4mm3 spatial resolution needed for depicting the aortic wall and 8-second temporal resolution, which is necessitated for DCE modeling.

Results: High-resolution motion-compensated DCE images were obtained in three healthy volunteers and two AAA patients. Image quality was superior to state-of-the-art alternative methods described in the literature. The vessel wall and aneurysm could be depicted and analyzed.

Conclusion: We developed a DCE protocol for AAA patients. With this protocol, we will now investigate the potential of DCE as a biomarker for indicating the progression of AAAs.

Poster Abstract 80

Presenter: Wei Su

Heart Failure & Arrhythmias

The Role Of Desmin Variants In Genetic Atrial Fibrillation

Wei Su, Stan W. van Wijk, Tyler J Kirby, Bianca JJM Brundel

Physiology Location Vumc

Atrial fibrillation (AF) is a the most common supraventricular tachyarrhythmia with uncoordinated atrial electrical activation patterns. The currently prevalence of AF in adults is between 2% and 4% in people >65 years. It has been recognized that in 15% of the AF population, AF is familial.

Most patients with DES variants reveal conduction defects and AF. Desmin is a classical type III intermediate filament protein encoded by the DES gene. The important function of desmin in the cardiac conduction system is related to the property of desmin to form networks that connect and anchor various cellular structures and organelles, Z-bands, to the cytoskeleton. Patients with DES gene S13F, N342D, and R454W variant often are diagnosed with AF at a younger age (<50).

Desmin aggregation within cardiomyocytes is the most significant histopathological hallmark of desmin cardiomyopathies. we analyzed this DES mutation in vitro by cell transfection experiments in combination with confocal microscopy. Of note, desmin-p.N342D forms desmin aggregates in transfected HL-1 cells, desmin-p.S13F and desmin-p.R45W forms normal desmin. The ratio of p.N342D aggregation 24.05%.

Poster Abstract 81

Presenter: Xiaoqing Sun

Pulmonary Hypertension & Thrombosis

Abl inhibition improves endothelial repair by enhancing VEGFR2 Y1175/ERK signaling

Xiaoqing Sun, Wenjun He, Xiaoke Pan, Anton Vonk-Noordergraaf, Harm Jan Bogaard, Jurjan Aman

Pulmonology Location Vumc

Rationale: Accumulating evidence points out an important role of rarefaction of the pulmonary microvasculature in emphysema. Vascular maintenance and stability critically depend on the vascular Endothelial Growth Factor Receptor 2 (VEGFR2). In a recent case report, the tyrosine kinase inhibitor imatinib resulted in fast clinical improvement of a patient with emphysema through an unknown mechanism. Here we explored the possible effect of imatinib on endothelial maintenance related to VEGFR2 signalling.

Methods: We evaluated the effect of imatinib on VEGFR2 signalling in human pulmonary microvascular endothelial cells (MVECs) and human umbilical vein endothelial cells (HUVECs).

Results: Compared to 0.1% DMSO treated cells, imatinib (0.5, 1, 2, 10μM) increased the phosphorylation of VEGFR2 at tyrosine Y1175 in a dose dependent manner. Consistently, imatinib activated Erk1/2, the downstream of VEGFR2 Y1175, in a dose dependent manner. Further experiments with 10μM imatinib revealed that VEGFR2 Y1175-Erk1/2 signalling was activated by imatinib up to 48hr, with a peak effect between 2 to 8hr, while Y951-Akt signalling was unaltered. Interestingly, repeated imatinib treatment can reactivate Y1175-Erk1/2 signalling in ECs after 46hr. Upon imatinib treatment, the expression of VEGFR2 was reduced, and it was normalized or increased after 24hr. Moreover, imatinib increased the proliferation and wound healing in HUVECs and MVECs. The effect of imatinib on VEGFR2 Y1175 signalling was also observed with Abl1/2 knockdown, in which combined Abl1 and Abl2 knockdown had an additive effect over knockdown of Abl1 or Abl2 alone.

Conclusion: By inhibiting Abl1/2, imatinib can improve endothelial cell integrity by modulating VEGFR2 Y1175-Erk1/2 signalling. This finding suggests a connection between Abl and VEGFR2 signalling in endothelial cells, and Abl inhibition may be a promising target to enhance endothelial integrity and prevent microvascular rarefaction.

Poster Abstract 82

Presenter: Eszter Tóth

Pulmonary Hypertension & Thrombosis

SGLT-2 inhibitors for the treatment of idiopathic pulmonary arterial hypertension: a proof-of-concept study

E.N. Tóth1, E. Duijvelaar1, K. Yoshida1, M. van der Veerdonk2, L. Meijboom3, H.J. Bogaard1

Pulmonary Medicine Location Vumc

Intro: Pulmonary arterial hypertension (PAH) is a progressive disease characterized by obliteration of the pulmonary vasculature resulting right ventricular (RV) failure. In rat models of PAH induced by monocrotaline (MCT) and Sugen-hypoxia, administration of empagliflozin resulted in decreased pulmonary artery pressure and decreased mortality. Additionally, empagliflozin has been shown to decrease pulmonary artery pressures independent of loop diuretics in patients with left heart failure. The effects of empagliflozin in patients with PAH remain unknown.

Aim: Our objective was to assess the safety and therapeutic potential of empagliflozin in patients with PAH

Method: This is an ongoing open-label single-arm interventional proof-of-concept study. Patients received daily dose of 10mg empagliflozin and are followed during a 12-week period. The primary study endpoints comprise study feasibility, drug safety and drug tolerability. Secondary endpoints are assessed with health-related quality of life (HRQoL) questionnaires, laboratory testing, 6-minute walk distance (6MWD), transthoracic echocardiography (TTE), and cardiac MRI (cMRI).

Results: Out of the planned total of 8 patients, 6 have already been enrolled over a 6-months screening period. During the study, two serious adverse events occurred. Both adverse events afflicted the same patient and were deemed to be unrelated to empagliflozin. Empagliflozin was well-tolerated by all enrolled patients. There were no differences in cardiac function, 6MWD and NT-proBNP levels in the patients who have completed the first 12-week follow-up period. Although patients reported increased energy levels, HRQoL questionnaires did not show significant improvement after 12 weeks of empagliflozin use. Data from additional participants is pending.

Conclusion: Empagliflozin appears to be safe and well-tolerated in patients with PAH. Although the study was not designed to establish clinical efficacy, there were no signals for clinical improvements.

Poster Abstract 83

Presenter: Eszter Tóth

Pulmonary Hypertension & Thrombosis

Unaffected BMPR2 mutation carriers have lower cardiac volumes and higher right ventricular cirumferential strain than healthy non-carriers

E.N. Tóth, L. Celant, S. Jansen, L.Meijboom, F. de Man, A. Vonk Noordegraaf, H.J. Bogaard

Pulmonary Medicine Location Vumc

Introduction: Pulmonary arterial hypertension (PAH) is a progressive disease of the pulmonary vasculature leading to right ventricular (RV) failure. PAH patients carrying a BMPR2 mutation have a more impaired RV function compared to non-carriers despite similar afterload. The presence of a pathogenic gene mutation could lead to RV impairment even in the absence of disease. Cardiac magnetic resonance imaging (CMRi) may provide insight to early changes in right ventricular structure and function.

Method: 29 BMPR2 mutation carriers (mean age 42.69 ± 15.42 yrs , 58% female) and 20 healthy controls (mean age 43.1 ±17.58 yrs, 45% female) underwent CMRi as part of an annual screening program. Images were analyzed to assess cardiac features and compared with healthy controls.

Results: BMPR2 mutation carriers had lower indexed RV end diastolic (64.8±14.33 ml/m2 vs 79.54 ± 17.55 ml/m2 ; p=0.004), end systolic (27.82± 8.44 ml/m2 vs 34.16 ± 10.51 ml/m2 ; p=0.034) and left ventricular end diastolic volumes (59.65 ± 2.73 ml/m2 vs. 68.65 ± 14.13 ml/m2; p=0.023) than healthy control subjects. Myocardial strain analysis at baseline demonstrated an increased global right ventricular circumferential strain (-15.74± 2.73 % vs. -12.72 ± 2.5% ;p= 0.001) in BMPR2 mutation carriers.

Conclusion: BMPR2 mutation carriers do not exhibit impaired RV function but show evidence of a differing cardiac architecture. This is possible evidence of an early cardiac adaptation to changes in the pulmonary vasculature, which has yet to be confirmed.

Poster Abstract 84

Presenter: Fenna Tuijnenburg

Heart Failure & Arrythmias

Long term follow-up of patients with a SCN5A loss-of-function variant

Fenna Tuijnenburg, Virginnio M. Proost, Christiaan C. Veerman, Saskia N. van der Crabben, Sander A.J.A. Groffen, Connie Bezzina, Arthur A.M. Wilde, Ahmad S. Amin.

Experimental Cardiology Location AMC

Background: Consensus on the appropriate treatment and follow-up of asymptomatic SCN5A variant carriers is missing since results regarding risk stratification are contradictory. Consequently asymptomatic carriers still frequently visit the outpatient clinic even though many patients remain asymptomatic. Therefore, the aim of this study is to investigate electro- and echocardiographic differences over time and to establish the cardiac event rate in SCN5A loss-of-function variant carriers.

Methods: This is a retrospective cohort study in patients with a SCN5A loss-of-function variant. We compared data of diagnostic tests at baseline to latest follow-up visit and analysed occurrence of cardiac events during follow-up in both symptomatic and asymptomatic carriers. Patients with a gain-of-function variant, variant of uncertain significance, no complete data available or a follow-up time of less than one year were excluded.

Results: During a median follow-up of 8.7 years (IQR 4.3-12.8), 323 SCN5A carriers were included of whom 273 were asymptomatic. The overall cardiac event rate was 5.6% but 24% in symptomatic and 2.2% in asymptomatic patients. After comparing baseline to follow-up data in asymptomatic patients, it shows a significant increase in PQ and QRS interval ($p < 0.001$). A significant decrease was found in resting heart rate during both electrocardiogram ($p<0.001$) and Holter ($p=0.005$) and in maximum heart rate during Holter ($p=0.037$) and exercise test ($p<0.001$). There was no significant difference in any of the evaluated echocardiogram parameters.

Conclusion: Overall, there was a significant increase in PQ and QRS interval during electrocardiogram at follow-up. The remaining significant differences are most likely caused by aging of the cohort. The overall cardiac event rate per year was 5.6%.

Poster Abstract 85

Presenter: Floor van den Dolder

Heart Failure & Arrhythmias

Western diet or aging do not aggravate hypertrophic cardiomyopathy disease progression in a heterozygous MYBPC3 c.2373insG mouse model

Floor van den Dolder, Edgar Nollet, Valentijn Jansen, Vincent Warnaar, Ali Nasser, Erik Bakker, Ed Eringa, Bram F. Coolen, Gustav J. Strijkers, Diederik Kuster, Jolanda van der Velden.

Physiology Location Vumc

Hypertrophic cardiomyopathy (HCM), the most common inherited cardiac disease, is characterized by hypertrophy and impaired relaxation. HCM is caused by mutations in genes encoding sarcomere proteins, but large heterogeneity in disease onset and severity suggests that second hits are needed for disease development. Aging and obesity are recognized as risk factors in patient cohorts that may contribute to phenotypic expression of HCM. To elucidate the mechanisms through which aging and perturbed metabolic health-related stress trigger development of HCM, we tested whether Western Diet (WD) feeding or aging could induce HCM in mice heterozygous (HET) for the Dutch founder mutation MYBPC3 c.2373insG.

MYBPC3+/2373InsG wild type (WT) littermates received WD for 10 weeks or were aged for 9/18-months. Mice underwent cardiac magnetic resonance imaging. Subsequently, the heart, epicardial fat, liver, and femoral arteries were isolated for further analysis. Initial data are reported below.

In mice exposed to WD, both WT and HET mice exhibited increased body weight and liver enlargement. Neither WD-feeding nor aging induced cardiac hypertrophy in MYBPC3+/2373InsG mice. When examining mitochondrial function, WD-fed mice, regardless of their genotype, displayed a minor decrease in NADH-linked respiration and a modest increase in leak respiration. Age-related increases in total oxidative phosphorylation capacity and respiration in response to fatty acids were evident at younger age in HET mice (9-months) compared to WT mice (18-months). When assessing the peripheral vascular response to acetylcholine, no significant differences were observed between genotypes, irrespective of diet or aging. Aged mice showed increased vascular wall thickness, reduced wall-to-lumen ratio and decreased response to acetylcholine independently from the genotype.

Western diet induced increased body weight, liver enlargement, and decreased mitochondrial function. Aging induced increased oxidative phosphorylation capacity, vascular remodeling and dysfunction. Overall, our initial data indicate that WD or aging do not aggravate disease progression in this HCM mouse model.

Poster Abstract 86

Presenter: Samira van Knippenberg

Microcirculation

Evaluation of Cardiovascular and All-cause Mortality after elective infrarenal repair of the Abdominal Aortic Aneurysm: A Systematic Review and Meta-analysis

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Vascular Surgery Location AMC

Background: Patients with an Abdominal Aortic Aneurysm (AAA) have a poor survival after elective endovascular aneurysm repair (EVAR) or open surgical repair (OSR), compared to the general population. This excess mortality is generally related to an increased cardiovascular risk. While short-term outcomes have been extensively studied, there is limited research on the long-term cardiovascular mortality. This systematic review and meta-analysis aimed to assess the pre-operative cardiovascular comorbidities and long-term incidence of cardiovascular and all-cause mortality following elective, infrarenal AAA-repair.

Method: A systematic search was conducted in Pubmed, Embase and COCHRANE databases. All studies published between January 2013 and May 2023, with ≥5 years mean follow-up, focusing on the all-cause and cardiovascular mortality after elective, infrarenal OSR and EVAR were included. Data on pre-operative cardiovascular comorbidities and mortality after AAA-repair were extracted. Weighted means of pre-operative cardiovascular comorbidities were assessed. Futhermore, a weighted linear regression analysis was performed to determine the annual incidence of all-cause and cardiovascular mortality 5 years after AAA-repair.

Results: 19 studies (84.144 patients) with a mean follow-up time of 68,9 (±13,3) months were included in the study; 8 studies were included for meta-analysis focusing on the cardiovascular mortality rate. The most common pre-operative cardiovascular comorbidities were hypertension (79,6%), dyslipidemia (42,6%) and coronary artery disease (26,5%). At 5 years, the mean all-cause mortality was 26,4% and the cardiovascular mortality 10,24%, with an estimated annual increase of 6,5% and 2,5%, respectively ($R(2)=0.801$, $P<0.001$ and $R(2)=0.891$, $P<0.001$). Patient undergoing EVAR had a significant higher incidence of cardiovascular-related mortality, compared to the OSR-group (B-coefficient: 1,925, $P<0.001$).

Conclusion: AAA patients exhibit a high prevalence of cardiovascular comorbidities, with elevated long-term all-cause mortality and cardiovascular mortality related following elective, infrarenal AAA-repair. These findings highlight the need for improved cardiovascular risk management, to minimize the long-term incidence of cardiovascular mortality in patients after AAA-repair.

Poster Abstract 87

Presenter: Daniel van Raalte

Diabetes & Metabolism

SGLT2-inhibitor treatment decreases kidney oxygen consumption in adults with type 2 diabetes: a randomized clinical trial using 11C-acetate PET imaging

Anne Hesp, Lars Snel, Ronald Boellaard, Merle Krebber, Jaap Joles, Daan Touw, Patrisch Schrober, Lothar Schwarte, Petter Bjornstad, Daniel van Raalte

Internal Medicine Location Vumc

Kidney hypoxia has been proposed as key pathophysiological mechanism in the development of chronic kidney disease. Hypoxia results from a mismatch in oxygen delivery and oxygen consumption. Sodium glucose cotransporter-2 inhibitors (SGLT2i) are kidney protective drugs that initially lower GFR. We hypothesized that SGLT2i lower kidney oxygen consumption by reducing glomerular hyperfiltration and associated tubular workload.

Twenty adults with type 2 diabetes (T2D) [sex 80% male, age 65 ± 12 years, BMI 30.1 ± 4.2 kg/m2, HbA1c 7.1 ± 0.9%, eGFR 72 ± 17 mL/min/1.73m2] received a 4-week treatment with SGLT2i ertugliflozin (ERTU) and matched placebo (PLB) in a randomized, double-blind cross-over study. Participants were treated with metformin and received maximal tolerable dose of an angiotensin receptor blocker. Whole-kidney oxygen consumption (k2) was measured by positron emission tomography using carbon-11 acetate. GFR was measured by gold-standard iohexol clearance. Tubular sodium transport (TNa) was calculated by kidney sodium load ([arterial Na]*mGFR) – urinary sodium excretion. Kidney efficiency equals TNa/k2.

Measured GFR was lower during ERTU (94 ± 14 mL/min) versus PLB (99 ± 15 mL/min) treatment (p=0.02). K2 was 0.086 ± 0.006 min-1 during ERTU and 0.091 ± 0.009 min-1 during PLB (p<0.01). Kidney sodium load (12.9 ± 1.90 vs 13.7 ± 2.22 mmol/min) and TNa (12.7 ± 1.87 vs 13.6 ± 2.18 mmol/min) were lower during ERTU (p=0.02 for both), while urinary sodium excretion (p=0.1) and TNa/k2 remained unchanged (p=0.7). TNa was strongly related to k2 (r=0.504; p<0.0001). Changes in K2 by SGLT2 treatment were driven by changes in insulin sensitivity as assessed during oral glucose tolerance test, and not by changes in reabsorbed sodium load.

In conclusion, we demonstrate that SGLT2i decreases mGFR and TNa, which is paralleled by lower kidney oxygen consumption, which could translate in lower hypoxia risk. Although cortical sodium transport is most ATP-effective, whole-kidney efficiency was not declined during SGLT2i treatment. Improvements in kidney substrate metabolism may drive the reductions in kidney oxygen consumption.

Poster Abstract 88

Presenter: Stan van Wijk

Heart Failure & Arrhythmias

Lamin C variants induce arrhythmia in Drosophila melanogaster

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Physiology Location VUmc

Introduction: Atrial fibrillation (AF), the most common progressive cardiac rhythm disorder, is associated with serious complications such as stroke and heart failure. Although common risk factors frequently underlie AF onset, in ~15% of the affected population, AF may have a genetic cause. Several AF families carrying variants in cytoskeletal proteins and in particular the nuclear protein Lamin A/C (LMNA) have been identified. How LMNA variants trigger AF is unknown.

Methods: To elucidate the effect of Lamin A/C variants on cardiac arrhythmicity, Drosophila melanogaster with equivalent mutations in the Drosophila Lamin C (LamC) gene, an orthologue of human LMNA were utilized. Heart wall movements of LamC variant Drosophila prepupa were recorded before (BTP) and after tachypacing (ATP). M-mode kymographs were made to analyze the heart rate (HR), arrhythmicity index (AI) and fractional shortening (FS). Furthermore, Drosophila were treated with microtubule affecting drugs Taxol (50nM), or colchicine (25µM) and the bromodomain and extraterminal protein inhibitor inhibitor RVX-208 (250µM) to study the underlying mechanism.

Results: Lamin C wild type (wt), ΔN, and R205W show a significant reduction in HRATP, but AIATP is not affected. In contrast, Lamin C variants N210K and R264Q show a significant reduction in HRATP and increased AIATP. None of these lines show a significant difference in FS. Pharmacological intervention with RVX-208 and Taxol significantly prevents reduction in HRATP, specifically in R264Q. Moreover, Taxol shows an antiarrhythmic effect in N210K, but is pro-arrhythmogenic in R264Q.

Conclusion: These results indicate that the Lamin C variants N210K and R264Q have a cardiac arrhythmogenic effect in Drosophila prepupa. The arrhythmogenic effect of the LamC variant was prevented by the microtubule stabilizing drug Taxol in N210K, but aggravated in R264Q. This indicates that Lamin C variants trigger various molecular pathways that drive arrhythmogenic effects.

Poster Abstract 89

Presenter: Rada Veeneman

Heart Failure & Arrhythmias

Bidirectional relations between depressive and anxiety symptoms and cardiovascular health in a clinical cohort

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Genetic Epidemiology Location AMC

Introduction: People with stress, anxiety, and depressive disorders are at increased risk of developing cardiovascular disease (CVD), but the mechanisms underlying these associations are poorly understood. It is particularly unclear whether or not there are causal relationships, and if so, in which direction: do mental health problems causally increase the risk of (more severe) CVD and/or does CVD lead to mental health problems?

Methods: We will study the relation between mental health problems and CVD in the Netherlands Study of Depression and Anxiety (NESDA; Nbaseline = 2981). NESDA includes individuals who have been diagnosed with a depressive or anxiety disorder and followed up for many years with detailed assessments. We will conduct two sets of analyses. First, we will examine metabolic syndrome components across three consecutive waves, allowing us to track their temporal patterns in relation to potential mediators. Employing structural equation modelling (SEM) we will construct intricate longitudinal models. Specifically, we will fit random intercept cross-lagged panel models. Second, we will concentrate on examining CVD endpoints over an extended period, with data spanning up to nine years. Logistic regression analysis will be used to assess the long-term effects of mental health and CVD diagnoses, along with potential mediators.

It has become increasingly recognized that men and women differ in their risk factors and treatment responses for CVD, as well as in their presentation of mental illness. Therefore, our study also aims to examine potential differences between men and women, to explore if health care interventions should be tailored to address the distinct needs of men and women.

Results: Currently, this project is in the data cleaning stage, with the analyses starting soon.

Conclusion: This comprehensive study, employing sophisticated modeling techniques, promises to enhance our comprehension of the intricate relationship between depressive and anxiety symptoms and cardiovascular disease.

Poster Abstract 90

Presenter: Yosta Vegting

Atherosclerosis & Ischemic Syndromes

Classical monocyte-derived and SPP1 lipid-associated macrophages orchestrate inflammatory and fibrotic processes in ANCA-associated glomerulonephritis

Yosta Vegting, Aldo Jongejan, Annette E Neele, Nike Claessen, Gal Sela, Koen H. M. Prange, Jesper Kers, Joris J T H Roelofs, Sandrine Florquin, Johan W van der Heijden, Onno de Boer, Ester Remmerswaal, Liffert Vogt, Frederike J Bemelman, Menno P J de Winther, Perry D Moerland, Marc L Hilhorst

Nephrology Location AMC

Kidney macrophage infiltration is one of the main histological hallmarks of vasculitic lesions and is strongly linked to disease activity in ANCA-associated glomerulonephritis (AGN). The precise contributions and mechanisms by which kidney macrophages influence local inflammation and long-term damage are still being explored. Here we investigate kidney macrophage diversity and functions using single-cell transcriptome analysis of freshly isolated kidney cells. We identified a novel Osteopontin (SPP1) lipid-associated kidney macrophage (SPP1 LAMs) subtype exhibiting distinctive and pronounced upregulation of fibrotic gene sets. Furthermore, 2 monocyte-derived macrophage (MDMs) clusters and C1Q resident-like macrophages (Res-like C1Q Mac) were identified, among which classical MDMs showed specific enrichment of inflammatory pathways. Evaluating kidney macrophage subsets revealed an increased proportion of classical MDMs, Res-like C1Q Mac, and SPP1 LAMs in AGN. Classical MDMs likely drive glomerular inflammation by profound release of IL1β and attraction of neutrophils and monocytes. Res-like C1Q Mac and SPP1 LAMs are attracted to inflamed glomeruli for the regulation of inflammation and SPP1 LAMs coordinate fibrotic processes. Targeting these specific macrophage subsets may potentially reduce long term kidney injury in AGN.

Poster Abstract 91

Presenter: Caitlin Vink

Atherosclerosis & Ischemic Syndromes; Diabetes & Metabolism

Reduced Microvascular Blood Volume as a Driver of Coronary Microvascular Disease in Patients With Angina and Non-obstructive Coronary Artery Disease: Design and preliminary data of the MICORDIS Study

Caitlin E.M. Vink, Elize de Jong, Tim P. van de Hoef, Steven A.J. Chamuleau, Yolande Appelman, Ed C. Eringa**

Cardiology and Physiology Location VUmc

Background: Ischemia with non-obstructive coronary arteries (INOCA) is part of the ischemic heart disease spectrum, and is particularly observed in women. INOCA has various mechanisms, such as coronary vasospasm and coronary microvascular dysfunction (CMD). A decreased coronary flow reserve (CFR) and-or increased myocardial resistance (MR) are commonly used to diagnose CMD. However, CFR and MR do not describe all pathophysiological mechanisms underlying CMD. Increased myocardial oxygen consumption (MVO2) normally increases myocardial blood volume (MBV), independently from myocardial blood flow (MBF). In addition insulin enhances MBV in healthy skeletal muscle, and this effect is impaired in INOCA-related conditions such as diabetes and obesity. Therefore, we propose that MBV is reduced in INOCA patients.

Aim: To assess whether myocardial blood volume (MBV) is decreased in INOCA patients, at baseline, during hyperinsulinemia and during stress.

Design: The MICORDIS-study is a single-center observational cross-sectional cohort study (identifier NTR7515). The primary outcome is MBV, compared between INOCA patients and matched healthy controls. The patient group will undergo coronary function testing using a Doppler guidewire, intracoronary adenosine and acetylcholine to measure CFR and coronary vasospasm. Both the patient- and the control group will undergo myocardial contrast echocardiography (MCE) to determine MBV at baseline, during hyperinsulinemia and during stress. Subsequently, cardiac magnetic resonance (CMR) will be evaluated as a new and noninvasive diagnostic tool for CMD in INOCA patients. Microvascular endothelial function is a determinant of MBV and will be evaluated by non-invasive microvascular function testing using EndoPAT and NO production will be measured.

Results: The preliminary data will be available and shown during the ASC-conference.

Poster Abstract 92

Presenter: Winnie Vos

Atherosclerosis & Ischemic Syndromes

T cell specific deletion of Casitas B lineage lymphoma-b reduces atherosclerosis by enhancing T cell exhaustion

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Medical Biochemistry Location AMC

Atherosclerosis is a lipid-driven inflammatory disease of the arterial wall, and the underlying cause of the majority of cardiovascular diseases. Recent advances in high-parametric immunophenotyping of immune cells indicate that T cells constitute the major leukocyte population in the atherosclerotic plaque. The E3 ubiquitin ligase Casitas B-lymphoma proto-oncogene-B (CBL-B) is a critical intracellular regulator that sets the threshold for T cell activation, making CBL-B a potential therapeutic target to modulate inflammation in atherosclerosis. We previously demonstrated that complete knock-out of CBL-B aggravated atherosclerosis in Apoe-/- mice, which was attributed to increased macrophage recruitment and increased CD8+ T cell activation in the plaque. To further study the T cell specific role of CBL-B in atherosclerosis, Apoe-/-CD4creCblbfl/fl (CBL-BCD4cre) mice and wild-type littermates (CBL-BWT) were fed a high cholesterol diet for ten weeks. CBL-BCD4cre mice had smaller atherosclerotic lesions in the aortic arch and root compared to CBL-BWT, and a substantial increase in CD3+ T cells in the plaque. Plaque necrotic core, macrophage content, and smooth muscle cell content were unchanged, while collagen content was decreased. Mice lacking T cell CBL-B had a 1.4-fold increase in CD8+ T cells as well as 1.8-fold increase regulatory T cells in the spleen. Both CD4+ and CD8+ splenic T cells had increased expression of CXCR3 and IFN-g, indicating a T helper 1 (Th1)-like/effector CD8+ T cell-like phenotype. Moreover, CBL-B deficient T cells had increased expression of PD-1 and TIGIT, indicating an exhausted T cell phenotype.

In conclusion, CBL-BCD4cre mice have reduced atherosclerosis, as a result of enhanced T cell exhaustion.

Poster Abstract 93

Presenter: Esther Vriend

Atherosclerosis & Ischemic Syndromes

Predictive Value of Cardiovascular Risk Factors on Cerebral White Matter Lesions and Brain Volume: The HELIUS Study

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Vascular Medicine *Location AMC*

Background and aim: Cardiovascular risk factors have been associated with early declines in brain volume and the development of white matter lesions (WML). However, previous studies on the association between cardiovascular risk factors and brain atrophy and cerebral WML were predominantly conducted in older European descent populations and were cross-sectional in design. In this study, we aim to investigate the predictive value of cardiovascular risk factors for cerebral WML and brain volumes in middle-aged participants using data from a large, multi-ethnic, population-based cohort.

Methods: We conducted brain magnetic resonance imaging (MRI) scans on participants from the HEalthy Life in an Urban Setting (HELIUS) study. We used 3T MRI sequences, including MP-RAGE, T2-weighted images, and FLAIR scans, to determine total brain volume, gray matter, white matter and WML volume, using automatic segmentation pipelines. WML volume values were log-transformed. Linear regression analyses, adjusted for age, sex, ethnicity, and cardiovascular risk factors at baseline, were performed to assess the predictive value of (change in) cardiovascular risk factors on brain volume and WML volume.

Results: MRI data were available in 562 participants of Moroccan, South-Asian Surinamese, and Dutch descent. At baseline, the mean age was 58.5 (range 35 - 65 years), 45% was female. The median follow-up time between the baseline visit and the MRI assessment was 8.4 [IQR 7.4; 9.5] years. Total brain volume was associated with baseline BMI, change in BMI, HbA1C, and the use of glucose-lowering medication, whereas WML volume was associated with cholesterol levels, use of antihypertensive medication, change in BMI, systolic BP, and a positive history of cardiovascular disease.

Conclusions: Cardiovascular risk factors demonstrate a longitudinal association with both brain volume and WML volume. These findings underscore the importance of considering cardiovascular risk factors in mid-life as predictors of future risk of cerebro- and cardiovascular diseases.

Poster Abstract 94

Presenter: Mengnan Wang

Microcirculation; Heart Failure & Arrhythmias

Empagliflozin prevents oxidative stress in human coronary artery endothelial cells via the NHE/PKC/NOX axis

Xiaoling Li, Mengnan Wang, Jan-Ole Kalina, Benedikt Preckel, Markus W. Hollmann, Martin Albrecht, Coert J. Zuurbier, Nina C. Weber

Anesthesiology Location AMC

Background: Sodium glucose co-transporter 2 inhibitor Empagliflozin (EMPA) ameliorates reactive oxygen species (ROS) generation in human endothelial cells (ECs) exposed to 10% cyclic stretch (pathological stress). Our previous studies have demonstrated that EMPA attenuates ROS generation by inhibiting the activity of sodium hydrogen exchanger 1 (NHE1) in ECs subjected to Tumor Necrosis Factor (TNF) -α or enhanced stretch. Pathological stretch is speculated to activate protein kinase C (PKC), activating nicotinamide adenine dinucleotide phosphate oxidase (NOX) and promoting ROS production in human ECs. We hypothesized that EMPA inhibits stretch-induced NOX activation and ROS generation through preventing PKC activation.

Methods: Human coronary artery endothelial cells (HCAECs) were pre-incubated for 2 h with either vehicle or EMPA in the presence or absence of the PKC inhibitor LY-333531 and the NHE-1 inhibitor cariporide, followed by exposure to cyclic stretch (5% (control) or 10% (stress)). PKC-β and NCX1 were silenced by siRNA .PKC activity, NOX activity, ROS production and intracellular calcium (Ca2+) were detected.

Results: Compared to 5% stretch, 10% stretch significantly increased PKC activity (5+Veh: 1.07±0.23 vs 10+Veh: 4.03±0.57, P<0.05). Treatment with EMPA and LY-333531 effectively inhibited the stretch-induced increase in PKC activity (10+EMPA: 0.83±0.40, 10+LY: 0.47±0.17, P both<0.05 vs 10+Veh). EMPA and LY-333531 also reduced NOX activity and ROS production in HCAECs exposed to 10% stretch. PKC-β knockdown blocked the NOX activation and ROS induction by 10% stretch. NCX1 knockdown prevented the increase in PKC activity caused by 10% stretch. Moreover, cariporide prevented the increase in PKC activity induced by 10% stretch (5+Veh: 1.07±0.23, 10+Cari: 1.90±0.30, P both <0.05 vs 10+Veh: 2.72±0.74), and the PKC inhibitory effect of cariporide was not augmented when combined with EMPA.

Conclusion: EMPA reduced NOX activity via attenuation of the NHE/PKC axis, leading to less ROS generation in HCAECs exposed to 10% stretch.

Poster Abstract 95

Presenter: Qian Wang

Atherosclerosis & Ischemic Syndromes

Malonate protection against acute infarction in isolated mouse hearts is lost in the presence of insulin

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Anesthesiology Location AMC

Background & Objectives: Malonate, an inhibitor of mitochondrial succinate dehydrogenase (SDH), is a promising novel protectant against acute ischaemia-reperfusion injury (IRI) through reduction of ischaemic succinate accumulation and ROS production during early reperfusion. However, numerous studies demonstrated that the metabolic milieu could affect the potency of cardioprotective treatments. Therefore, in this study, we explored whether and how the severity of cardiac I/R injury and cardioprotection by malonate is affected by the metabolic milieu. We hypothesized that protection by malonate is affected by metabolic milie.

Methods: For detecting the cardioprotection of malonate, isolated Langendorff-perfused hearts (n=8 to 19 per group) of C57Bl/6N mice were perfused with perfusate to which substrates and hormones were added in a step-wise manner: 1) glucose (G), 2) glucose + glutamine (GG), 3) glucose + glutamine + palmitate (GGP), or 4) glucose + glutamine + palmitate + insulin (GGPI), and subjected to 30 min ischaemia (I) and 90 min reperfusion (R). Heart were treated with malonate (5mM) or vehicle (saline) during the first 2.5 min of reperfusion only. For measuring metabolic intermediates including lactate, succinate and glucose-6-phosphate (G6P), metabolomics analysis was performed using hearts subjected to 30 min ischemia or 30 min ischemia and 7 min reperfusion with vehicle/malonate treatment in GG, GGP and GGPI group. Data are presented as mean ± SD.

Results: Going from glucose-only perfusate to a perfusate containing all examined substrates and hormones, the severity of cardiac I/R injury increased: infarct size (%) went from 31.9 ± 10.9% to 37.7 ± 10.6% to 52.3 ± 14.2%, and finally to 68 ± 9.9 %. Similar trends for increased IRI were observed for LDH, End-Diastolic Pressure (EDP) and recovery of the Rate-Pressure Product (RPP). When compared with vehicle control, malonate significantly decreased IS% (control vs malonate: 31.9 ± 10.9% vs. 23.3 ± 4.8%, 37.7 ± 10.6% vs. 20.9 ± 8.5%, 52.3 ± 14.2% vs. 40.2 ± 9.2 %), decreased LDH (0.87 ± 0.26 vs. 0.54 ± 0.36 U/min/GWW, 1.01 ± 0.42 vs. 0.5 ± 0.26 U/min/GWW, 2.06 ± 0.76 vs. 1.28 ± 0.17U/min/GWW)and increased the RRP recovery (64.1 ± 16.6 %vs. 83.3 ± 17.9 %, 55.9 ± 11% vs. 72.3 ± 4%, 39.3 ± 17.7% vs. 55.9 ± 8.3%) in the G,GG and GGP group. The EDP was significantly decreased by malonate in the G and GG group (32.8 ± 14.3 mmHg vs. 20.9 ± 9.8 mmHg, 35.1 ± 8.7 mmHg vs. 22.2 ± 8.6 mmHg). Most importantly, adding insulin to the perfusate nullified protection by malonate for all four outcomes. Metabolic results showed that, end-ischemc succinate almost doubled when adding palmitate (0.79±0.17 vs. 1.29±0,25), but not further increased with insulin

(1.29±0,25 vs. 1.43±0.28). However, insulin did increase end-ischemic lactate (2.80±1.47 vs. 4.4±1.46) and G6P (1.66±2.98 vs. 3.39±2.88).Indicating that the loss of malonate protection is associated with increased lactate that can act as a competitive inhibitor of cellular malonate uptake, or attributed to the increased G6P which could induce hexokinase 2 detachment. However, compared with control group, after 7 min of reperfusion, the succinate(0.24±0.10 vs. 0.21±0.06, 0.43±0.12 vs. 0.46±0.15, 0.53±0.17 vs. 0.39±0.10), lactate(0.58±0.31 vs. 0.32±0.11, 0.50±0.21 vs. 0.60±0.46, 0.60±0.29 vs. 0.44±0.28), and G6P(0.46±0.67 vs. 0.24±0.03, 0.38±0.25 vs. 0.39±0.44, 0.97±0.86 vs. 0.76±0.64) did not significantly changed by malonate in GG, GGP and GGPI group.

Conclusions: The severity of cardiac I/R injury is lowest with glucose-only perfusate, and increased with increased complexity of the metabolic milieu. Although malonate is a strong protectant against cardiac IRI for most metabolic milieus, in the isolated heart, protection is lost in the presence of insulin. The loss of malonate protection might associated with increased lactate and G6P, which will be furtherly investigated in the follow-up study.

Poster Abstract 96

Presenter: Vincent Warnaar

Heart Failure & Arrhythmias

Effect of Mavacamten on the myofilament-mitochondrial axis in hypertrophic cardiomyopathy with and without sarcomere mutation

Vincent Warnaar, Ali Nassar, Edgar Nollet, Michelle Michels, Diederik Kuster, Jan W. Buikema, Jolanda van der Velden

Physiology Location Vumc

Hypertrophic cardiomyopathy (HCM) is the most common autosomal dominant inherited form of cardiomyopathies. A lack of knowledge about the key regulators that are involved in the disease onset and progression results in limited therapeutic strategies to prevent or cure HCM. This study focuses on the role of mitochondrial (dys)function in the pathophysiology of HCM, and test the effect of the myosin inhibitor Mavacamten (MAVA). Previous research has shown that MAVA corrects the so-called Super Relaxed (SRX) / Disorded-relaxed (DRX) ratio of myosin. MAVA shifts myosin heads from the high energy consuming DRX to an energy-saving SRX state. To discern both acute and chronic effects of MAVA on the myofilament-mitochondrial axis during HCM disease progression we combine studies in human cardiac tissue samples, cultured human tissue slices, a cell-derived human muscle model and a HCM mouse model.

To study MAVA-mediated preventive effects on cardiac remodeling in a human muscle model, we generate EHT made of stem cell-derived cardiomyocytes harboring the MYBPC3 (c.2373insG) mutation and control lines. Next, ex vivo tissue slices, of cardiac samples from HCM patients, will be kept in culture to study the reversibility of the altered myofilament-mitochondrial axis by chronic MAVA treatment. Cardiac samples from patients with obstructive HCM will also be used to study the acute effect on myosin head composition and mitochondrial function. To link the in vitro data on the myofilament-mitochondrial axis with in-depth in vivo characterization of the heart we make use of HCM mice with the MYBPC3 (c.2373insG) founder mutation. Functional readouts such as contraction and relaxation parameters are obtained and subsequent analyses include mitochondrial function, SRX/DRX ratio analyses. It is expected that MAVA reduces ATP demand and improves mitochondrial function by normalizing the SRX/DRX ratio. Subsequent improved mitochondrial function, and the coincident reduced oxidative stress, exerts positive effects on cardiac remodeling.

Poster Abstract 97

Presenter: Janneke Woudstra

Microcirculation

Association between Coronary Vasomotor Function Measured via Invasive Coronary Function Testing and Peripheral Vasomotor Function

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Cardiology Location Vumc

Introduction: Coronary vasomotor dysfunction (cVMD), an important underlying cause of non-obstructive coronary artery disease (ANOCA), consists of coronary vasospasm and coronary microvascular dysfunction (i.e. endothelial dysfunction (CED) and/or endothelium-independent dysfunction (CEID)) and is clinically assessed by invasive coronary function testing (ICFT). In this study we evaluated vasomotor function in the skin as a non-invasive correlate of cVMD.

Methods: 35 ANOCA patients underwent ICFT for the assessment of cVMD, consisting of intracoronary acetylcholine (ACh) and adenosine, with Doppler flow measurements. Current guidelines were used to define cVMD. Cutaneous microvascular function was assessed using Laser Speckle Contrast Analysis in the forearm, combined with endothelium-dependent vasodilator ACh and endothelium-independent vasodilator sodium-nitroprusside (SNP). The reactive hyperemia index (RHI) was assessed by EndoPAT.

Results: Patients (80% women) had a mean age of 59 ± 9 years. cVMD was observed in 28 patients, including 23 with coronary vasospasm, 20 with CED and 4 with CEID, with overlapping endotypes. Patients with and without cVMD had a similar RHI (2.0±0.5 vs 1.8±0.4; p=0.342). In contrast, cVMD was associated with lower peripheral perfusion responses to ACh and SNP (14814.2±8361.5 vs 22394.9±5757.7 perfusion units (PU); p=0.043 and 21208.5±14159.3 vs 33423.7±6305.6 PU; p=0.003).

Conclusion: Coronary vasomotor dysfunction is highly prevalent in ANOCA patients. These patients have a lower peripheral response to ACh and SNP, while no difference in RHI is seen. This study is the first to provide evidence for the potential value of the peripheral vasomotor function as a non-invasive tool for detecting cVMD.

Poster Abstract 98

Presenter: Carmen Yap

Atherosclerosis & Ischemic Syndromes

Creation and characterization of novel MFS mouse models

Carmen Yap, Myrthe Hoogeland, Eline Mol, Vincent Wakker, John Spijkers, Nanda van Eeken, Vincent Christoffels, Vivian de Waard

Medical Biochemistry *Location AMC*

Marfan Syndrome (MFS) is one of the most common connective tissue disorders, due to mutations in the gene fibrillin-1 (FBN1). The encoded glycoprotein is a fibrillary extracellular matrix protein that gives structure and elasticity to the connective tissues. Therefore, mutations in FBN1 either lead to dysfunction (dominant negative) or reduced amount (haploinsufficient) of fibrillin-1, resulting in defective connective tissue. Without any surgical intervention it can be lethal due to aortic aneurysm formation, dissection and rupture. As FBN1 is a large gene, many possible mutations the 65 exons can occur, of which most will lead to a phenotype that is identified as MFS. Yet, only few different MFS animal models have been studied so far. Using CRISPR/Cas9 technology we generated various novel MFS mouse models and investigated whether these models recapitulate the typical MFS phenotype as observed in the conventionally used MFS mouse model, the Fbn1C1041G/+ mouse. Histological measurements showed that four out of five of the novel MFS models had increased aortic root dilatation, and three out of five had an enlarged ascending aorta. In two out of five MFS lines, there was an increased wall area/thickness. Of the non-cardiovascular features, one MFS line showed an increased body weight and especially the dominant negative lines had enhanced signs of scoliosis. An additional test was performed to explore whether the expression of inflammation/fibrosis genes in the lungs were relevant as a method for further validation of these novel MFS mouse models. Preliminary results show increased expression of inflammation and fibrosis genes in all tested MFS lines.

In conclusion, we successfully generated novel MFS mouse models to be used for future MFS syndrome research.

Poster Abstract 99

Presenter: Dominic Zimmerman

Heart Failure & Arrhythmias; Atherosclerosis & Ischemic Syndromes

Genetic Factors Predisposing to Sudden Cardiac Arrest

Dominic Zimmerman, Najim Lahrouchi, Doris Milosavljevic, Rafik Tadros, Christian Krijger, Virginnio Proost, Sean Jurgens,Leander Beekman, Charlotte Glinge, Veronica Dusi, Lia Crotti, Peter Schwartz, Jacob Tfelt-Hansen, Arthur AM Wilde, Michael WT Tanck, ESCAPE-NET Investigators, Connie R Bezzina, Hanno Tan

Experimental Cardiology Location AMC

Sudden cardiac arrest (SCA) is a major health problem in the affluent world, accounting for 15–20% of all natural deaths in adults in the USA and Western Europe, and for up to 50% of all cardiovascular deaths. SCA is due to cardiac causes that result in cardiac arrhythmia (ventricular fibrillation, VF). SCA is lethal within minutes if left untreated and survival rates are presently only 5-20%. In this project we aim to discover which genetic factors predispose an individual to SCA. To identify genetic factors that contribute to risk of SCA we conducted a genome wide association study (GWAS) that investigated the role of common genetic variants of small effect size. Using the cohort ARREST (n = 3841) as SCA cases and LifeLines (n = 17085) as general population controls, we performed a GWAS which found a single novel locus that was under the statistical significance threshold of p-value: 5x10e-8. This locus was found on chromosome 1 (rs117251079, p-value: 1.12463e-08, OR: 2.99071, surrounding CHGB, TRMT6, MCM8). Following this association analysis, we performed a meta-analysis of our original GWAS with 3 similar cohorts to increase statistical power, namely AGNES, GEVAMI and PREDESTINATION which are 3 European ancestry case-control sets consisting of individuals with VF (cases) and general population (controls) during a first STEMI prior to revascularization. From this meta-analysis another statistically significant novel locus was found, on chromosome 11 (rs4148646, p-value: 4.222e-08, surrounding KCNJ11, ABCC8. We then performed genetic correlation analyses with associated traits (e.g., Type 2 Diabetes, Coronary Artery Disease, Hypertrophic Cardiomyopathy), and polygenic risk scores. Future work will also include WGS/WES (whole genome/exome sequencing)-based rare variant association studies that investigate the role of rare variants of larger effect that are associated with SCA, specifically in younger individuals.

Poster Abstract 100

Presenter: Sabrina Zwetsloot

Atherosclerosis & Ischemic Syndromes

Protocol for the development of a core outcome set for intermittent claudication: a systematic review and Delphi study

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Vascular Surgery Location Vumc

Introduction: Currently, it is unknown which patients suffering from peripheral arterial disease (PAD) and an abdominal aortic aneurysm (AAA) will experience disease progression or develop adverse cardiovascular events. VASCUL-AID is an European Horizon-sponsored research project that aims to build an AI-driven user interface to adequately predict PAD and AAA progression. This tool will help patients alter their own disease course by offering tailored feedback. As a first step in this multi-year project, clinically relevant and patient-reported outcomes need to be identified. Literature on PAD is heterogeneous and lacks standardization. Therefore, the main objective of the current study is to create consensus among patients, clinical specialists and researchers on a list of core outcomes that should be applied to all future research on intermittent claudication (IC).

Methods: The study will be conducted in three phases; the first being a systematic review identifying all outcomes related to conservative, endovascular, and operative treatment of IC (Fontaine classification II and III); the second a three-step Delphi study stakeholders to create a list of outcomes regarded as most important; the third an expert panel meeting finalizing the definitive core outcome set (COS). Stakeholders will include patients (n=40) and vascular specialists (n=40) from each of our European participating centers.

In developing this COS, we adhere to the Core Outcome Set-STAndards for Development (COS-STAD) recommendations and the Core Outcome Measures in Effectiveness Trials (COMET) Handbook. The COS will be published in accordance with the COS-STAndards for Reporting statement. For the Delphi study, recommendations as reported by Sinha et al. will be complied with.

The final COS will consist of a maximum of ten outcomes. These outcomes will be utilized for the consecutive retrospective and prospective studies.