Science Exchange Day

Abstract booklet

27 SEPTEMBER 2019

Amstel Lecture Theatre
VU University medical centre

Preface

Dear fellow scientists,

The central highlight of the Science Exchange Day 2019 is the poster session during which the informal contacts between researchers can blossom. Like in earlier years, we are proud to enable the presentation of 50 posters based on corresponding abstracts, showing exciting results from the research performed at all the research institutes. In this booklet, you can read all the 50 selected best abstracts and find the corresponding numbers of their bulletin boards in the poster area.

The posters are displayed the entire day in the Foyer of location VU University Medical Center. They are ordered by similarity in research approach rather than by disease or discipline to enable maximal interaction. During the coffee breaks there is time to roam the Foyer for interesting posters and fill in the scavenger hunt questionnaire to have a chance to win a very convenient prize!

Finally, each presenter will pitch his or her poster in one minute in front of the audience and judges. This will hopefully result in the exchange of ideas and new collaborations to further strengthen our research. The three best posters with their accompanying abstracts will also be awarded a prize.

On behalf of the Science Exchange Day 2019 organizing committee,

Shanice Beerepoot, Alan Jenks, Joyce Kors, Lena Sialino & Dorit Verhoeven

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Abstracts

Fundamental studies

Poster 1

The Role of the LXR-EEPD1 axis in Cholesterol Metabolism

Author and department: Suzanne A.E. van Wouw, Department of Medical Biochemistry

Coauthors: R. Ottenhof, J. Kingma, N. Zelcer

Introduction: We have recently reported that Endonuclease/Exonuclease/Phosphatase family Domain containing 1 (EEPD1) is a target of the sterol responsive nuclear Liver X Receptor (LXR) in macrophages. In both human and rodent macrophage-like cell lines EEPD1 was required for maximal ABCA1-mediated cholesterol efflux towards ApoAI. The aim of this study is to further characterize the physiological role of EEPD1 in lipid metabolism.

Methods: To study the physiological role of EEPD1 we used CRISPR/Cas9-based methodology to generate EEPD1-KO mice. We are evaluating the function of EEPD1 specifically in bone marrow-derived macrophages (BMDM), and also in whole-body lipid metabolism. In BMDMs we studied cholesterol efflux and distribution, the inflammatory response, profiled the transcriptional landscape using RNAseq and used lipidomics to investigate the lipid profile of the cells. To investigate the role of EEPD1 in systemic lipid and energy metabolism EEPD1-KO and control mice are being challenged with a high fat diet (HFD).

Results: In BMDMs we found that EEPD1-deficient macrophages have decreased maximal cholesterol efflux to ApoA1, supporting our previous report. Furthermore, we observed that intracellular distribution of cholesterol in EEPD1-deficient macrophages is distinct from that observed in control macrophages. This observation may be related to our finding using RNAseq that the expression of cholesterol biosynthetic genes was reduced in EEPD1-deficient macrophages. The lipidomic results show an increase in cholesterol esters and decrease of triglycerides in KO BMDM. Our in vivo results from the HFD experiment suggest that EEPD1 KO mice are more susceptible to weight gain than WT mice following a HFD and the existence of a difference in the plasma triglyceride levels between these two mouse lines.

Conclusion: Our ongoing experiments further support a role for EEPD1 in regulation of cellular and systemic lipid homeostasis.

Poster 2

Novel protective role of FHL2 deficiency in diet-induced Type 2 Diabetes

Author and department: Jayron Habibe, Department of Medical Biochemistry

Coauthors: M. Clemente, M. Vos, M. Nieuwdorp, T. Scheithauer, H. Herrema, D. van Raalte, E. Eringa, C.J. de Vries

Introduction: Type 2 diabetes (T2D) is a prevalent metabolic disease. The mechanisms underlying T2D remain unknown, however many factors contribute to its development including high bodyweight. T2D is characterized by pancreatic β-cells becoming incapable of meeting insulin demands due to peripheral insulin resistance. Four and a half Lim Domains 2 (FHL2) functions as a mediator for intracellular signaling and increased FHL2 methylation is associated with T2D. Our hypothesis is that high FHL2 levels increase the risk of T2D development through modulating β-cell function or peripheral insulin sensitivity. Here, FHL2 was studied using wild type (WT) and FHL2-/- mice on chow and High-Fat Diet (HFD).

Methods: Chow and HFD-fed mice were placed in metabolic cages to determine caloric intake, energy expenditure and exercise. Oral Glucose Tolerance Test (oGTT) was performed to determine glucose clearance, and Insulin Tolerance Test (ITT) was performed to determine insulin sensitivity. Afterwards, the mice were sacrificed and organs harvested for immuno-histochemical/-fluorescent staining. Pancreatic islet isolation via collagenase digestion was performed to evaluate Glucose- stimulated Insulin Secretion (GSIS) between WT and FHL2-/- isolated-islets. Gene expression analysis was performed on isolated-islets via RT-qPCR.

Results: Food consumption of FHL2-/- and WT mice on chow and HFD was similar, whereas FHL2-/- mice exhibited reduced weight gain. Oral Glucose Tolerance Test (oGTT) revealed improved glucose clearance and insulin secretion in FHL2-/- mice. Insulin Tolerance Test (ITT) depicted comparable insulin resistance between groups. Harvested mouse pancreases underwent immunohistochemistry/ immunofluorescence and quantification revealing higher insulin and GLUT2 protein expression in FHL2-/- mice. GSIS-assay of isolated-islets showed enhanced insulin secretion by FHL2-/- islets. RT-qPCR revealed increased MafA expression in FHL2-/- islets.

Conclusion: Our results indicate reduced diet-induced T2D in FHL2-/- mice involving enhanced insulin secretion. Based on this we propose that FHL2 deficiency lessens T2D and that individuals with high FHL2 expression may be at risk to develop T2D.

Poster 3

No changes of the neuroinflammatory protein YKL-40 are observed in post-mortem brain of cases with Frontotemporal Lobar Degeneration and Alzheimer's Disease

Author and department: Yanaika S. Hok-A-Hin, Department of Clinical Chemistry

Coauthors: D. Wouters, L. Boonkamp, J.M. Hoozemans, C.E. Teunissen, M. Del Campo

Introduction: Evidence indicates that neuroinflammation plays an important role in the development of dementias such as Frontotemporal lobar degeneration (FTLD) and Alzheimer's disease (AD). YKL-40 (Chitinase 3-like I), a glycoprotein involved in inflammatory process, is increased in cerebrospinal fluid (CSF) of FTLD and AD patients, suggesting YKL-40 as a potential CSF biomarker reflecting ongoing neuroinflammatory process. We hypothesize that CSF YKL-40 levels reflect YKL-40 changes in the brain. Therefore, we aimed to thoroughly analyze YKL-40 expression in post-mortem tissue of non-demented controls and dementia cases with different pathologies (FTLD and AD) and its correlation with ante-mortem CSF YKL-40.

Methods: YKL-40 was analyzed by immunohistochemistry in paraffin-embedded sections from post-mortem frontal cortex of FTLD (n=23) and non-demented controls (NDC, n=7) as well as temporal cortex from AD (n=52) and NDC (n=51). YKL-40 was analyzed in post-mortem lysates by Western blot (frontal cortex from FTLD (n=46), AD (n=5) and NDC (n=9)) and ELISA (frontal cortex from FTLD (n=67) and NDC (n=14)), temporal cortex from AD (n=7) and NDC (n=11)). CSF YKL-40 was measured in a subset of cases from which both ante-mortem CSF and post-mortem frontal cortex tissue was available (n=9).

Results: YKL-40 levels in post-mortem brain tissue was similar between non-demented controls and FTLD or AD by either immunohistochemistry, Western blot, or ELISA ($p>0.05$). No correlation between ante-mortem CSF YKL-40 and post-mortem brain YKL-40 was observed ($p>0.05$). Interestingly, strong YKL-40 immunoreactivity in the cerebral vessels was observed in post-mortem temporal cortex of AD cases with cerebral amyloid angiopathy (CAA).

Conclusion: The significant CSF YKL-40 changes described previously are not observed in post-mortem brain tissue. These data suggest that CSF YKL-40 changes do not reflect the involvement of YKL-40 in neuroinflammation in the brain or that changes on this protein occur transiently. The strong YKL-40 reactivity observed in CAA may indicate a relationship of brain YKL-40 with vascular (dys)function in AD.

Poster 4

Differential receptor use of Enterovirus D-68 strains in human airway epithelial cultures

Author and department: Lance Mulder, Department of Clinical Virology

Coauthors: A. Sridhar, E. Karelehto, J. Baggen, L. van der Linden, A. Meijer, F. van Kuppeveld, D. Pajkrt, K. Wolthers

Introduction: Enterovirus D-68 (EV-D68) is an emerging respiratory pathogen in the family Picornaviridae that can cause severe respiratory disease and has neurotrophic potential resulting in "polio-like" paralysis, particularly among children. Recent work has shown that EV-D68 uses α2,6- and α2,3-linked sialic acid as a receptor to infect cells. Furthermore, several EV-D68 strains have also been shown to use a non-sialylated receptor for infection in sialic acid deficient cell lines. It is unclear whether sialic acid independent strains circulate in the human population and if the use of an alternative receptor confers any additional benefits in terms of pathogenesis or tropism of EV-D68.

Methods: In order to further elucidate EV-D68 pathogenesis in a physiological human infection model, we used human airway epithelial (HAE) cultures. HAE cultures are fully differentiated ex vivo models that contain different cell types of the airway epithelium including mucus producing and cilia beating cells.

Results: We report that HAE cultures obtained from three different biological donors are permissive for infection by three clinical isolates of EV-D68 (2042, 947, and 1348) at 37˚C. All strains exhibited similar replication kinetics over a 72h time period. In terms of receptor usage of EV-D68, 2042, the isolate most closely resembling the prototype Fermon strain, was dependent on sialic acid while clinical isolates 947 and 1348 retained sialic acid binding capability but were able to use an alternative receptor (heparan sulfate) for infection as reported previously in various cell lines. We also identified by immunofluorescence imaging that ciliated cells are the primary targets for EV-D68 infection with no visible infection of mucus producing cells or p63+ Basal cells at 8h and 72h time points.

Conclusion: We are currently performing cytokine analysis of the media samples to assess the variation in host response to the different EV-D68 strains. Our findings provide insight into EV-D68 infection, receptor usage, and cell tropism in human airway epithelium.

Poster 5

Recombination Dynamics in Enterovirus Species C

Author and department: Lieke Brouwer, Department of Medical Microbiology

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Introduction: Poliovirus, member of Enterovirus species C (EV-C), is almost eradicated due to vaccination policies. However, outbreaks of vaccine derived PVs (VDPVs) have been reported. These VDPVs are recombinants of the PV vaccine and other EV-C strains. Yet, little is known about recombination in this species. The aim of our present research was to study recombination dynamics in EV-C.

Methods: A database was constructed of full length EV-C study sequences from a Malawian study cohort, and full length EV-C sequences publically available in GenBank. Maximum Likelihood (ML) trees were constructed of the VP1, 2C and 3DPol regions. A segregation analysis was performed on the entire genome, and groups were identified by analysing the distribution of 3DPol and VP1 pairwise p-distances.

Results: The phylogenetic trees and segregation analysis of EV-C show phylogenetic violation in the nonstructural parts of the genome. Plots of the 3DPol and VP1 pairwise p-distance showed four distinct groups (I, II, III and IV) and clusters in both CVA21 and CVA24.

Conclusion: We saw phylogenetic violation within EV-C, implying recombination. We hypothesize that the division of the strains in four groups in 3DPol can be explained by tropism, as the groups infect either the gastro-intestinal (I, IV) or the respiratory tract (II, III), and possibly different cell types within these organ systems. Tropism would also explain the cluster in CVA24, as these strains come from conjunctivitis patients.

Poster 6

An enhancer cluster controls gene activity and topology of the *SCN5A-SCN10A* locus *in vivo*

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Introduction: Mutations and variations in and around *SCN5A*, encoding the major cardiac sodium channel, influence impulse conduction and are associated with a broad spectrum of arrhythmia disorders including Brugada syndrome. Genome-wide association studies have identified common variants in the *SCN5A-SCN10A* locus influencing ECG parameters (conduction). These variants are mainly located in non-coding, putative transcriptional regulatory elements. Here, we identified an evolutionary conserved regulatory element cluster with super enhancer characteristics downstream of *SCN5A*, which drives localized cardiac expression *in vivo* and contains conduction velocity-associated variants.

Methods: CRISPR/Cas9 genome editing tools were used to generate a series of deletions of the enhancer cluster in the mouse genome. Transcriptome analysis of the ventricles from the enhancer cluster deletion mice was performed using RNA-sequencing. The enhancer cluster deletion mice were subjected to *in vivo* and *ex vivo* ECG recordings, and optimal mapping. The three-dimensional organization of the *Scn5a-Scn10a* locus was investigated using 4C-sequencing.

Results: We demonstrate that deletion of the enhancer cluster and its individual elements cause a decrease in *Scn5a* expression and conduction slowing. Furthermore, deletion of the cluster caused a marked change in the three-dimensional conformation of the locus, involving a reduction in contacts between other regulatory elements and promoters. Surprisingly, RNA-sequencing showed that the enhancer cluster is selectively required for cardiac *Scn5a* expression. Homozygous enhancer cluster deletion mice are embryonic lethal, but still express low levels of *Scn5a*, suggesting the enhancer complex is partially redundant during development.

Conclusion: Our studies reveal physiological roles of an enhancer cluster in the *SCN5A-SCN10A* locus, show that it controls the conformation of the locus and *Scn5a* expression, and suggest genetic variants affecting its activity may influence cardiac function.

Poster 7

Mapping the immune system in Alzheimer's disease

Author and department: Lynn van Olst, Department of Molecular Cell Biology and Immunology

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Introduction: Age-related brain diseases like dementia are laying an increased burden on our modern society. In order to establish novel treatments, deeper understanding of these age-related brain diseases is needed. Alzheimer's Disease (AD) is the most prevalent form of dementia and is currently affecting nearly 44 million people worldwide with a high unmet medical need. Multiple evidence suggests a link between the (peripheral) immune system and AD development. Although the role of microglia cells is at the moment heavily investigated, peripheral immune cells have got only minor attention. Recently, the use of time-of-flight cytometry (CyTOF) used for high-dimensional single-cell mapping of the immune system has boosted immunology research in different fields. Until now, this potential of full spectrum cellular characterization has not yet been exploited to explore and understand the dynamics of both peripheral and central immune cells during AD progression.

Methods: To broaden our knowledge about the role of the immune system in AD, we are currently using a panel of 37 cellular protein markers combined with single-cell mass cytometry to make a comprehensive, detailed map of the immune system of 250 Alzheimer patients and controls from the Amsterdam Dementia Cohort. To investigate time-dependent and location-dependent changes in the immune profile during the progression of AD, we study isolated immune cells of the brain, spleen and blood from the APP/PS1 Alzheimer mouse model at different time-points.

Results: N.A.

Conclusion: N.A.

Poster 8

Pulmonary arterial endothelial cells from chronic thromboembolic pulmonary hypertension patients show enhanced platelet adhesion

Author and department: Xue Manz, Department of Pulmonology

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Introduction: Chronic thromboembolic pulmonary hypertension (CTEPH) is characterized by obstructive thromboembolic material in pulmonary arteries despite anti-coagulation therapy. Increased *in situ* thrombosis is postulated to play a role in the pathogenesis. We hypothesize that the pulmonary endothelium is involved in the formation of local blood clots and investigated whether endothelial cells from CTEPH patients provide a pro-thrombotic environment.

Methods: Freshly isolated platelets of healthy individuals were perfused over pulmonary artery endothelial cells (PAECs), isolated from pulmonary endarterectomy material of CTEPH patients or control lung lobectomy tissue. Perfusion was applied with shear rates of 2.5 dyn/cm^2. Platelet adhesion was promoted by endothelial activation with histamine (1 µM, 30 minutes).

Results: Activation of CTEPH-PAECs with histamine resulted in a significant 1.5-fold increase in platelet adhesion compared to control-PAECs (24.95±6.93% vs. 17.98±4.87%, p=0.03). Gene expression of von Willebrand Factor (vWF) was 5-fold increased resulting in a 1.5-fold higher vWF secretion by CTEPH PAECs upon histamine stimulation (0.93±0.51 nM vs. 0.62±0.54 nM, p=0.049). Pre-treatment of platelets with an antibody against vWF (CLB-Rag35) reduced platelet binding on CTEPH-PAEC in a concentration-dependent manner (1-100 µM), while an increase in concentration above 1 µM did not have an additional effect on platelet inhibition in controls. The presence of fresh thrombi in histological sections of pulmonary arteries coincides with high in vitro platelet adhesion, while PAECs from CTEPH patients with organized thrombi exert platelet adhesion comparable to controls.

Conclusion: Our study demonstrates increased platelet adhesion on histamine activated CTEPH-PAECs in a vWF-dependent manner. Enhanced platelet adhesion may be an important initiator of increased *in situ* thrombosis in CTEPH, which we were able to target with a direct platelet inhibitor. Importantly, the correlation of histological evidence and functional experimentation indicates that patients with fresh thrombi might benefit the most from vWF-targeted treatment.

Poster 9

eNOS regulates VEGF-dependent transcytosis in primary human endothelial cells

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Introduction: Endothelial cells form the inner lining of the vasculature, functioning as a barrier between the blood and the underlying tissue. Rather than being a passive layer of cells, the endothelium actively senses its environment, dynamically changes accordingly, and produces a variety of vasoactive mediators. One such mediator is the gaseous molecule nitric oxide (NO), which is widely known as a potent vasodilator. It is evident that NO produced by endothelial NO synthase (eNOS) plays a key role in vascular homeostasis, including maintaining a proper barrier function of the endothelium.

Methods: Previous work has shown that eNOS is essential for the induction of vascular permeability by a variety of inflammatory mediators, including vascular endothelial growth factor (VEGF). However, how eNOS mediates VEGF-induced permeability remains incompletely understood. So far, it is evident that eNOS regulates VEGF-mediated changes in the junctional network. However, whether eNOS also mediates VEGF-induced permeability via the transcellular pathway remains to be explored.

Results: Here, we show that inhibition of eNOS expression by siRNA reduces VEGF-induced permeability of a 766-da and 70-kda fluorescent tracer in human umbicial vein endothelial cells (HUVECs). These tracers are representative for paracellular and transcellular leakage, respectively. In addition, we show that the eNOS inhibitor L-NAME prevents VEGF-induced expression of PLVAP, a key protein involved in transcellular transport. In addition, knockdown of eNOS expression reduces basal expression levels of PLVAP and NRP2, as well as VEGF-induced upregulation of these transcripts.

Conclusion: This suggests that in addition to its effects on the paracellular pathway, eNOS also regulates permeability via the transcellular pathway.

Inhibiting extracellular vesicle release from breast cancer cells to combat drug resistance

Author and department: Caitrin Crudden, Department of Pathology

Coauthors: M. Bebelman, M. Jonk, B. Westerman, C.R. Jimenez, D.M. Pegtel

Introduction: Despite advances in detection and therapy, breast cancer remains the second leading cause of cancer-related death in women. A large proportion of patients that initially respond to therapy eventually relapse due to resistance mechanisms, that ultimately underpin disease mortality. Production of extracellular vesicles (EVs) is upregulated upon transformation, and has been shown to drive metastatic outgrowth and been linked to therapy resistance. It appears that cancer EV production may be a consequence of an altered metabolism, representing a new vulnerability.

Methods: To address whether selective inhibition of the pathways that drive EV production could sensitive breast cancer cells to therapy, we used paired sensitive/resistant cell sub-lines generated by incremental exposure to pharmaceuticals in vitro. We used HER2 overexpressing cell (BT-474) sub-lines resistant to HER2 monoclonal antibody trastuzumab, and HER2/EGFR tyrosine kinase inhibitor lapatinib, alongside a HER2 low/absent cell line (MCF-7) resistant to the chemotherapeutic paclitaxel. Employing bioluminescence assays to quantify EV release, glucose consumption and lactate secretion, we interrogated the links between EVs, metabolism and drug resistance.

Results: Our panel of breast cancer cell lines upregulate EV release, glucose consumption and lactate production, when compared to a non-tumorigenic breast epithelial control. Both EV release and aerobic glycolysis were upregulated in BT-474 sub-lines displaying resistance to both trastuzumab and lapatinib. Mechanistic links between the two systems are suggested by experiments showing that selective inhibition of EV release returns glucose consumption/lactate production to the levels of sensitive lines. Although the aerobic glycolysis rates appeared upregulated in the MCF-7 paclitaxel resistant sub-line, this mechanism of resistance did not translate into an upregulation of EV release.

Conclusion: Our results suggest that EV production appears linked to the mechanism of resistance underlying HER2 targeting, both by blocking antibody and tyrosine kinase inhibitors. In these instances, inhibition of EVs may represent a feasible approach to combat acquired resistance.

Poster 11

Tumor-induced alterations of the bone stroma as novel therapeutic targets for osteosarcoma treatment

Author and department: Manon Mulders, Department of Pathology

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Introduction: Osteosarcoma is a pediatric bone tumor characterized by high metastatic potential and poor prognosis. Defining the interactions between osteosarcoma and the complex bone microenvironment can provide insights into novel therapeutic strategies. We previously showed that osteosarcoma cells release extracellular vesicles (EVs) that “educate” mesenchymal stem cells (MSCs) to promote tumor growth and metastasis formation. We found that TGFβ bound to the surface of the cancer EVs causes this pro-tumorigenic effect by inducing IL6 expression in MSCs. We hypothesize that besides TGFβ on the surface of EVs, exosomal RNA within the EVs can bind Toll Like Receptor 3 (TLR3) in MSCs, contributing to the tumor-induced bone stroma alterations.

Methods: Using RNA sequencing we compared MSCs induced by osteosarcoma EVs vs. naïve MSCs in the presence or absence of a TGFβ inhibitor and analyzed with GSEA. To validate the cancer EV TGFβ independent effect on MSCs, we performed qPCR. To study the heterogeneous exosome population in vivo we generated osteosarcoma cells impaired in EV release by shRNA-mediated knockdown of proteins involved in the sorting and release of EVs.

Results: We found a variety of pathways induced in tumor-educated MSCs vs naïve MSCs. Blockade of TGFβ signaling demonstrated that, differently from IL6, the EV-mediated induction of multiple inflammatory pathways is TGFβ-independent. Many genes enriched in these pathways are TLR3 induced or signaling related. Knockdown of exosomal protein Rab11b in osteosarcoma cells results in less induction of IL6 in exosome treated MSCs, indicating that Rab11b might be involved in the sorting or release of TGFβ-bound EVs.

Conclusion: Our study suggests that multiple exosomal cargo molecules cause exosome-mediated alterations in the bone stroma involved in tumor progression and metastasis formation. These molecules present interesting novel targets for combinational therapy in osteosarcoma treatment.

Poster 12

Doxycycline Compromises Mitochondria in Preclinical Models of Abdominal Aortic Aneuryms

Author and department: Arnout Mieremet, Department of Medical Biochemistry

Coauthors: S. Wanga, R.C.I. Wust, R. Balm, R.H. Houtkooper, C.J.M. de Vries, J.H.N. Lindeman, V. de Waard

Introduction: Prevalence of abdominal aortic aneurysm (AAA) is high in the elderly population, with approximately 1 in 50 affected. Current treatment options are limited to surgical intervention. Therefore, there is an urgent need to develop pharmacological treatments to halt AAA progression. Convincing preclinical evidence was obtained for the antibiotic doxycycline in murine AAA models and in human AAA tissue, leading to the initiation of a clinical trial with doxycycline in Dutch AAA patients. However, this trial terminated prematurely because of increased rate of aortic growth in the treatment group. As doxycycline is known to also induce mitonuclear imbalance due to its inhibitory effect on mitochondrial translation, we aim to characterize the mitochondrial effect of doxycycline using complementary *in vitro*, *ex vivo*, and *in vivo* models.

Methods: The effect of doxycycline was examined in *in vitro* human aortic smooth muscle cell (SMC) cultures, *ex vivo* human AAA tissue, and *in vivo* murine aortic tissue for mitochondrial function and tissue repair capacity.

Results: Oral doxycycline treatment of male C57Bl6 mice for 3 weeks with doxycycline (500mg/kg/day) or control (ampicillin; 50mg/kg/day) leads to an increase in mitonuclear imbalance and impaired mitochondrial function in aortic tissue. This was confirmed in *ex vivo* treated human AAA tissue and *in vitro* SMC cultures. Moreover, doxycycline caused mitochondrial network fragmentation, reduced oxidative phosphorylation-mediated ATP production, reduced SMC migration and proliferation, and reduced hallmark SMC alpha-smooth muscle actin mRNA and protein expression.

Conclusion: Doxycycline affects the mitochondria of SMCs, thereby changing the SMC phenotype, which reduces the repair capacity of SMCs to combat cellular and tissue damage, as encountered in AAA. We obtained novel insights about the effects of doxycycline in SMCs, which is of importance in the design and validation of novel therapies to inhibit AAA progression.

Poster 13

Optimization of a small RNA sequencing protocol for sensitive and accurate detection of plasma-EV associated miRNAs.

Author and department: Monique van Eijndhoven, Department of Pathology

Coauthors: E. Aparicio, J. Pérez-Boza, T. Bradley, M. Hackenberg, M. Pegtel

Introduction: Accumulating evidence suggests that miRNAs associated with plasma derived extracelluar vesicles (plasma-EV) have potential as biomarkers. We recently published a proof of concept study for the use of plasma-EV miRNAs to monitor treatment in Hodgkin Lymphoma patients. Candidate miRNA biomarkers are often discovered using commercially available small RNA sequencing protocols, however it's proven difficult to validate these candidates by RT-qPCR or in a multicenter study. Therefor miRNAs are far from being used for diagnostic or clinical purposes. We hypothesize that this is due to severe biases introduced during library preparations for small RNA sequencing.

Methods: We aim to develop a small RNA sequencing protocol that is able to sensitively and accurately detect miRNAs. The adapters used in commercial protocols have a preference to ligate to certain sequences, we try to overcome this bias by designing adapters with the addition of random nucleotides (5N). Moreover we use these 5N as Unique Molecular Identifiers (UMIs, barcodes) to correct for PCR bias during amplification of the libraries. We test the novel 5N-adapters and protocol using several synthetic cell-miRNA spike-ins as well as RNA from plasma-EV from cancer patients.

Results: Our results show that the use of 5N-adapters reduces the ligation bias compared to commercial adapters, based on the distribution of an equimolar pool of n=30 synthetic cel-miRNA spike-ins. The use of UMIs improves the distribution even further. With 5N we can detect more different endogenous miRNAs (up to 1000) and we find that the miRNA profiles detected with 5N adapter differ a lot from the ones detected with a commercial protocol.

Conclusion: Overall our results show that using 5N-adapters to prepare libraries improves the sensitivity and accuracy of small RNA sequencing. If validated this may lead to the use of miRNAs as biomarkers in clinical practice.

Poster 14

Transcriptional activation of fucosyltransferase (FUT) genes using the CRISPR-dCas9-VPR technology reveals potent N-glycome alterations and increased tumor growth of colorectal cancer cells

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Introduction: Aberrant fucosylation in cancer cells is considered as a signature of malignant cell transformation and it is associated with tumor progression, metastasis and resistance to chemotherapy. Specifically, in colorectal cancer cells, increased levels of the fucosylated Lewisx antigen are attributed to the deregulated expression of pertinent fucosyltransferases, like fucosyltransferase 4 (FUT4) and fucosyltransferase 9 (FUT9). However, the lack of experimental models closely mimicking cancerspecific regulation of fucosyltransferase gene expression has, so far, limited our knowledge regarding the substrate specificity of these enzymes and the impact of Lewisx synthesis on the glycome and the tumor growth of colorectal cancer cells.

Methods: Therefore, we sought to transcriptionally activate the *Fut4* and *Fut9* genes in the well-known murine colorectal cancer cell line, MC38, which lacks expression of the FUT4 and FUT9 enzymes. For this purpose, we utilized a physiologically relevant, guide RNA-based model of de novo gene expression, namely the CRISPR-dCas9-VPR system. Induction of the Fut4 and Fut9 genes in MC38 cells using CRISPR-dCas9-VPR resulted in specific neo-expression of functional Lewisx antigen on the cell surface.

Results: Interestingly, Lewisx was mainly carried by N-linked glycans in both MC38-FUT4 and MC38-FUT9 cells, despite pronounced differences in the biosynthetic properties and the expression stability of the induced enzymes. Moreover, Lewisx expression was found to influence the overall N-glycome composition and resulted in significantly increased tumor growth of our MC38-glycovariants *in vivo*.

Conclusion: In conclusion, exploiting the CRISPR-dCas9-VPR system to study cancer-specific regulation of gene expression has broad application possibilities in oncology research.

Poster 15

The human gut organoid, a promising model to study enterovirus infection and disease pathogenesis

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Introduction: Enteroviruses (EVs) are a major source of human infections worldwide, with a broad spectrum of disease symptoms, from diarrhea and skin rash to more severe disease like meningitis and paralysis. Elucidating EV pathogenesis has been limited by the lack of suitable models that faithfully mirror normal human physiology and pathophysiology. Organoids are stem cell-derived in vitro 3D organ models and an excellent system that has potential for studying on EV-host interaction, virus evolution, and antiviral compound testing on a human system.

Methods: The 3D fetal gut organoids are an “inside out” representation of human physiology with the basal side on the outside facing the environment and the apical side facing the inwards. During culture, the proximal and distal organoids are “opened up” and cultured as a monolayer on transwell inserts to establish viral infection. The monolayers were apically exposed to enterovirus A71 (EV-A71) and subsequent viral replication was assessed by quantifying the production of viral RNA and virus replication at several time points over a course of six days.

Results: Using the monolayer transwell system we show that EV-A71 infects the epithelium monolayers from the apical surface. We will present data on infection of the monolayer model with EV-A71, cell tropism of the virus, and monolayer permeability after infection.

Conclusion: The human fetal gut derived intestinal organoid model is a powerful model for studying enterovirus infection and related disease pathogenesis. Continued development of the organoids cultures by including components of the normal host tissue microenvironment such as immune cells and blood vessels, will facilitate and simplify studies on human viral pathogenesis, and improve the development of platforms for pre-clinical evaluation of vaccines, antivirals and therapeutics.

Poster 16

Activated memory T cells cause NF-κB-dependent inflammatory activation of the endothelium: identification of novel therapeutic targets

Author and department: Kim C.M. Jeucken, Department of Rheumatology and Immunology

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Introduction: Endothelial cells (EC) are contributors to inflammation via expression of inflammatory mediators, which can be induced via canonical and NF-κB-inducing kinase (NIK)-dependent NF-κB signalling. Although the ligands activating NF-κB signalling are known, it is less clear which are the cellular sources.

Methods: $CD4^+CD45RO^+$ memory T cells were isolated from healthy PBMC and cultured in presence of anti-CD3 and anti-CD28 for 72h, after which supernatant was harvested. Endothelial cells were stimulated with 50% T_m supernatant (T_m sup) or brought into co-culture with T_m cells. After 72h protein and RNA of T_m sup stimulated EC was harvested followed by analysis of NF-κB signalling and expression of inflammatory mediators. Culture supernatants of T_m sup stimulated EC and co-cultures were analysed for inflammatory mediators. To repress canonical NF-κB signalling an inhibitor of IKKβ (iIKKβ) was used, to repress NIK-dependent NF-κB signaling an inhibitor of NIK (iNIK) was used.

Results: Stimulation with T_m sup led to activation of both NF-κB signalling pathways. After T_m sup stimulation EC showed increased mRNA levels of all tested inflammatory mediators. mRNA levels of chemokines, cytokines, and growth factors were significantly reduced after treatment with iIKKβ and iNIK. Additionally, treatment with iIKKβ led to a reduction in mRNA levels of adhesion molecules. Of note, treatment with either iIKKβ or iNIK led to a significant reduction of CXCL5 and IL6 in the culture supernatant of both T_m sup stimulated EC and co-cultures.

Conclusion: Our findings demonstrate that activated T_m cells can induce NF-κB-dependent inflammatory activation of EC. Targeting of NF-κB signaling via IKKβ or NIK reduces inflammatory activation of the endothelium and may be a potential novel therapeutic target.

Diagnostic efficacy of [18F]FDG PET/CT in staging low-intermediate grade, estrogen receptor positive breast cancer

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Introduction: Accurate staging of patients with primary breast cancer is essential for an optimal treatment plan. The current imaging standard for staging, positron emission tomography/computed tomography with [18F]Fluorodeoxyglucose ([18F]FDG PET/CT), might be insufficient for detection of distant metastases, specifically in low grade, estrogen receptor positive (ER+) breast cancer, due to lower metabolic activity. The aim of this study was to investigate the efficacy of [18F]FDG PET/CT in staging patients with low-intermediate grade, ER+ breast cancer.

Methods: 79 patients diagnosed with grade 1-2, ER+ clinical stage IIB/III or locoregional recurrent breast cancer were retrospectively included. Visual analysis was performed, comparing lesions detected on conventional imaging (mammography/ultrasound/magnetic resonance imaging/CT/bone scintigraphy) with lesions detected on [18F]FDG PET/CT. Pathology outcomes were considered as the gold standard. Tracer uptake in each suspect PET-positive lesion was (semi)quantified: volumes of interest were defined on the PET scan to determine standardized uptake values (SUVmax/SUVmean/SUVpeak), total lesion glycolysis (TLG) and metabolic tumor volume (MTV). These quantitative parameters were correlated with pathological features of tumors (histological subtype/grade/ER/PR/HER2 expression/mitotic activity index).

Results: Scans were analyzed visually and (semi)quantitatively. 476 lesions could be identified with all imaging modalities. Based on the gold standard, 194/476 (40.8%) lesions were interpreted as “true positive” (TP), 35/476 (7.4%) as “false positive” (FP) and 59/476 (12.4%) as “false negative” (FN) on [18F]FDG PET. “FP” and “FN” lesions were mainly located in the axilla region. The median SUVmax for “TP” and “FP” lesions was 3.7 (IQR: 2.3-5.7) and 2.7 (IQR: 1.9-4.6), respectively. For “TP” primary tumors SUVmax, SUVpeak, SUVmean correlated with histological subtype, showing higher uptake in ductal carcinoma compared to lobular carcinoma ($p < 0.05$).

Conclusion: These preliminary data indicate that [18F]FDG PET/CT might not correctly identify a substantial amount of lesions and therefore could lead to incorrect staging of patients with low grade, ER+ breast cancer.

Poster 18

Thalamic volume as a proxy for cognitive impairment: towards clinical application?

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Introduction: Thalamic atrophy occurs early in multiple sclerosis (MS) and relates strongly to cognitive impairment. However, it is unknown whether thalamic volume is able to predict the presence of cognitive disturbances in a clinical setting. The aim of the present study was to determine whether thalamic volume can be used to distinguish cognitively impaired (CI) from preserved (CP) patients in a real-life sample presenting with subjective cognitive complaints and to define the cut-off value for thalamic volume with the highest discriminatory value.

Methods: A total of 52 MS patients with cognitive complaints and 76 healthy controls (HCs) underwent tests for verbal and visuospatial memory, information processing speed and verbal fluency. CI was defined as ≥2 tests with scores ≤-2 standard deviations (SD) below the scores of HCs. All participants underwent MRI scanning at 3T. Thalamic volumes of MS patients were corrected for head size, adjusted for age and converted to z-scores. Receiver Operating Characteristic analysis was performed to determine optimal thalamic cut-offs.

Results: 23.1% of MS patients (12/52) were defined as CI. Thalamic volume was significantly lower in CI compared to CP patients (P<.001). The optimal thalamus volumetric cutoff was at -2.3 SD, at which 9/12 patients were correctly classified as CI and 28/40 were correctly classified as CP (i.e. sensitivity=75%, specificity=70%). Positive Predictive Value was 42% and Negative Predictive Value was 90% (NPV, area under the curve=0.77).

Conclusion: With a moderate sensitivity and specificity and a high negative predictive value, preservation of thalamic volume indicates that it is unlikely that objective cognitive disturbances are present. Taking into account more structural and functional measures and influencing factors such as cognitive reserve may improve the detection of cognitive impairment in MS.

Poster 19

Evidence based recommendations for cortical lesion imaging in multiple sclerosis

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Introduction: Cortical demyelinating lesions (CL) are clinically relevant in MS, but notoriously hard to visualize with MRI. Double inversion recovery (DIR) MRI has been shown to be more sensitive than standard T2-weighted and fluid-attenuated-inversion-recovery (FLAIR) sequences. However, DIR still only captures ~20% of CL. Recently, phase-sensitive inversion recovery (PSIR) was suggested to be more sensitive than DIR. However, this contention still lacks histopathological validation. This study compares all so far suggested sequences for CL imaging to each other and to histopathology.

Methods: Coronally cut brain slices of 23 MS patients were obtained. 2D-T1, 2D-PD/T2 weighted, 3D-FLAIR, 3D-DIR and 2D-PSIR sequences were acquired using a 3T whole-body scanner, after which 93 tissue blocks were sampled from these slices. All MR slices were scored in consensus (PMB/JJGG) for CL, blinded to histopathology. Subsequently, tissue samples were stained for myelin and scored for CL types I-IV. Histopathology scores were matched to MRI scores upon deblinding and sensitivity and specificity measures were calculated. Then, a retrospective scoring was performed.

Results: In a mixed model controlling for patients, DIR and PSIR detected more CL than T1, PD/T2 and FLAIR (51, 53, 20, 12 and 12 of 224, respectively; $p<.001$). Sensitivity was 8.9% (T1), 5.4% (PD/T2), 5.4% (FLAIR), 22.8% (DIR) and 23.7% (PSIR). DIR and PSIR did not differ from each other. Combining DIR and PSIR added 5% explained variance. Retrospective scoring increased sensitivity to 29.0% (T1), 23.7% (PD/T2), 23.2% (FLAIR), 42.4% (DIR) and 56.3% (PSIR). Specificity for T1, T2 and FLAIR was 80.0%, 91.1% for DIR and 88.3% for PSIR. Again, the difference between DIR and PSIR was not significant.

Conclusion: At 3T, advanced imaging sequences DIR and PSIR outperform conventional sequences viz. T1, PD/T2 and FLAIR. Moreover, DIR and PSIR are highly specific. Using DIR or PSIR, or –ideally- both is therefore recommended to assess CL in MS.

Poster 20

Different network functional connectivity characteristics of responders and non-responders to attention training in MS

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Introduction: Cognitive rehabilitation shows only moderate average group effects in MS patients. While cognitive impairment in MS is related to changes in functional connectivity (FC) of cognitive brain networks such as the default mode network (DMN), it is unknown how functional network characteristics affect the brain's ability to respond to cognitive training. We aimed to investigate whether baseline resting-state FC of the DMN, dorsal attention (DAN) and ventral attention (VAN) network can distinguish responders from non-responders to an attention training in MS.

Methods: Patients were randomized into an attention training (home-based computerized C-Car: 7-week, 45 min/week, N=58, age=48.4±10.2 years, 34 women, RRMS=42, median EDSS=4.0) or a waiting-list control group (CG, N=24, age=48.5±9.4 years, 19 women, RRMS=16, median EDSS=4.0). Neuropsychological assessments at baseline and follow-up included attention, memory, information processing speed, and executive functioning. Based on the CG, a practice-adjusted reliable change index (RCI) was calculated. Responders were defined as RCI>1.64 (90% CI) on ≥2 tests. 3DT1 MRI and resting-state fMRI was obtained. Within- and between-network FC of the DMN, DAN, and VAN were calculated using relative correlations and the Brainnetome atlas and compared between responders and non-responders.

Results: Responders (N=22) and non-responders (N=36) did not differ on any of the demographic and clinical variables (age, sex, education, MS subtype, EDSS, disease duration, GMV, WMV, baseline cognition). Responders, compared to non-responders, had lower average FC between DMN-DAN (0.85 vs 0.92 respectively; p=0.04) and DMN-VAN (0.90 vs 0.98 respectively; p=0.04). Responders' FC between DMN-DAN and DMN-VAN did not significantly differ from HC. Non-responders had higher FC between DMN-DAN (p=0.04) and DMN-VAN (p=0.003) compared to HC.

Conclusion: Lower FC between DMN and attention networks seems an indicator of response to attention training in MS, which might reflect a more intact baseline network functioning.

Poster 21

Sensitive and reproducible MEG resting-state functional connectivity measures in Alzheimer's disease

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Introduction: Cognitive dysfunction in Alzheimer's disease (AD) has been ascribed to disturbed functional communication between brain areas, which can be assessed with magnetoencephalography (MEG). However, analysis of functional networks in AD has been hampered by a lack of reproducible, yet sensitive, measures of functional connectivity (FC). This study aims to assess both the discriminatory value and replicability of the AEC-c and PLI, two FC measures insensitive to volume conduction, in two cohorts of AD versus control patients.

Methods: Patients with probable AD, with biomarker proof, and subjective cognitive decline (SCD) underwent a five-minute resting-state MEG measurement using a whole-head MEG scanner (Elekta Neuromag Oy). Data consisted of a test-(AD=40; SCD=40) and validation-(AD=17; SCD=17) cohort. Time series were estimated for 90 regions of interest (ROIs) in the automated anatomical labelling (AAL) atlas. For each of five frequency bands, the AEC-c and PLI were calculated between all 90 ROIs, and connections were averaged per ROI. The frequency-specific group averages in both cohorts were compared using permutation-testing (corrected using the false discovery rate).

Results: The AEC-c showed significantly lower global connectivity for the AD-group compared to the SCD-group, in the alpha (8-13Hz; mean(AD)0.52; mean(SCD)0.534; p=<.001) and beta (13-30Hz; mean(AD)0.514; mean(SCD)0.525; p=<.001) bands, for almost all 90 ROIs. In the delta band (0.5-4Hz), the AD-group showed significantly higher connectivity. For the SCD-group, the AEC-c measured higher AEC-c values in the posterior regions for the alpha-band. The PLI only revealed significant connectivity differences in the theta band (4-8Hz). These results could be replicated in the validation-cohort, except for the PLI-differences in the theta band.

Conclusion: The AEC-c is a sensitive and reproducible metric, able to distinguish significant FC differences in a cohort of AD and SCD patients. The results are suggestive for a decline in efficient communication between brain regions in AD.

Poster 22

Subcortical and cortical brain morphology associated with obsessive-compulsive symptoms in 2551 children from the general population

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Introduction: Obsessive-compulsive symptoms (OCS) are common in the general population, but it is unclear whether subclinical OCS symptoms and obsessive-compulsive disorder (OCD) are part of a neuroanatomical continuum. The goal of this study is to investigate the relation between OCS and subcortical and cortical morphology in a large population-based sample of school-aged children.

Methods: The study included 2551 participants, aged 9-12 years, from the population-based Generation R study (https://generationr.nl/). OCS were measured using the Short Obsessive-Compulsive Disorder Screener (SOCS). Structural (3T) MRI scans were processed using FreeSurfer to study intracranial volume, subcortical volumes, vertex-wise cortical thickness and surface area. We used linear regression models to investigate the association between severity of OCS with brain morphology. To emulate case-control comparisons from the literature we also compared children scoring above the clinical cut-off of the SOCS ('probable OCD' cases) with a matched symptom-free group.

Results: SOCS scores correlated negatively with intracranial volume. Vertex-wise analysis showed that OCS was associated with a thicker right inferior parietal cortex, but this relationship disappeared after adjusting for total behavioral problems. Probable OCD cases had larger thalami compared to symptom-free children, but this effect disappeared after correction for multiple comparisons.

Conclusion: We present the largest population-based neuroimaging study of pediatric OCS to date. OCS showed a stronger association with total intracranial volume than regional brain measures. Probable OCD cases showed similar thalamus alterations as previously reported in unmedicated pediatric OCD patients. The findings suggest a possible distinction between neural correlates of OCS at the subclinical and clinical level.

Patterns of learning preferences: A Q-methodological study into medical student learning

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Introduction: How students perceive their learning environment, and the degree to which it coincides with their preferences for teaching and learning, has been shown to influence outcomes like study success, motivation, retention and satisfaction. With an increasingly diverse medical student population, we should be mindful of changes in the teaching and learning preferences of students. Learning to identify these preferences opens up the possibility of creating more effective and appealing education for all students. For this study, we were interested in 1) exploring how students differ in their learning preferences; and 2) to determine the reasons for these differences.

Methods: Q-methodology is a mixed-method research design used for the systematic study of subjectivity. To start, we generated a set of 54 statements on learning preferences from the literature. Then in an individual interview, first year medical students rank ordered these statements from 'most agree' to 'most disagree'. Finally, the Q-sorts were analyzed using factor analysis. The factor solutions were evaluated, interpreted and finalized by three researchers on methodological, statistical, and qualitative criteria through consensus.

Results: A four-factor solution (i.e. profiles) was found to best fit the data collected from 52 students, and explained 52% of the variance. Students in profile 1 (N=15) can be summarized as 'Development-oriented', students in factor 2 (N=8) as 'Assessment-oriented', students in factor 3 (N=10) as 'Group-oriented', students in factor 4 (N=14) as 'Future practice-oriented'.

Conclusion: The identified student profiles describe distinct patterns of preferences for learning in tutorial groups. Fundamental differences between the profiles relate to their preferred methods of learning, pedagogical and didactical strategies of tutor teaching, and degree of student-centeredness of the learning environment. The results also indicate aspects of teaching and learning that are valued by all students, like the importance of a safe learning environment, feedback and collaborative learning.

Poster 24

PreSTOP: Patients' perspective on discontinuation of CML TKI-treatment

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Introduction: Chronic myeloid leukemia (CML) is a malignant hematologic disease with recommended life-long treatment with tyrosine kinase inhibitors (TKIs). Several trials show that some CML patients with stable disease can discontinue TKI-treatment without loss of efficacy. However, little is known about Dutch patients' perspective and attitude on, and willingness to discontinue TKI treatment and influencing factors. Objective: Gaining insight into patients' willingness and preferences regarding discontinuation of CML TKI-treatment and to identify possible influencing factors.

Methods: A cross-sectional, multicenter study using a questionnaire was conducted in the Netherlands. Adult CML patients were recruited. Patients were asked about their willingness and preferences regarding discontinuation of TKI-treatment. Logistic regression analysis was used to determine factors associated with patients' willingness to discontinue TKI-treatment.

Results: A total of 185 patients participated in this study. Most CML patients (79.5%) were willing to discontinue treatment. Patients most frequently reported: no more side effects, being afraid of an aggressive relapse, and being frequently monitored as the most important advantage, disadvantage, and condition for discontinuing treatment respectively. Univariate logistic regression showed that young age (2.47 (1.09-5.59) P = 0.03), paid work (3.04 (1.44-6.41), P = 0.00), being informed about discontinuation studies (6.25 (2.36-16,52) P=0.00), and severe adverse events (2.64 (1.21-5.76), P = 0.01) were associated with patients' willingness to discontinue TKI-treatment.

Conclusion: Most patients were willing to discontinue TKI-treatment and reported their preferences about this. Several factors were associated with TKI- treatment discontinuation. Our findings can be used to optimize and tailor patient information about TKI-treatment discontinuation.

Poster 25

Health-related quality of life of Dutch children with a chronic health condition aged 8 to 17 years

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Introduction: Children with chronic health conditions (CHCs) are at greater risk of having a lower health-related quality of life (HRQOL) than their peers. However, in the Netherlands, an overall picture of HRQOL of patients with CHCs as a group is missing. Therefore, this study aimed to investigate HRQOL of children with various CHCs and compare their scores with the general Dutch population. Variables associated with HRQOL were assessed.

Methods: 1209 children (8-17 years) with various CHCs, receiving care in the Emma Children's Hospital Amsterdam UMC, completed the Pediatric Quality of Life Inventory (PedsQLTM) on the KLIK website (www.hetklikt.nu). Six domain (physical and psychosocial health, emotional, social, and school functioning, and total score) and 23 individual item scores were compared with the general Dutch population (N=966, norm data collected in 2018) using Independent T-Tests and Chi-Square Tests. Linear Regression Analyses were performed to determine the association of sociodemographic and school variables with HRQOL.

Results: Children with CHCs reported lower HRQOL domain scores than the general Dutch population ($p \leq .001$, $d = .20$-$.88$), except for social functioning (figure 1a and 1b). They also reported more problems on almost all individual items than the general population ($p \leq .002$). Finally, age, gender and school absence were significantly associated with HRQOL scores ($p \leq .008$, $\beta = .10$-$.37$).

Conclusion: This study provided HRQOL data of children with various CHCs and the general Dutch population. It was shown that HRQOL of children with CHCs is substantially impaired. Structural monitoring of HRQOL in daily clinical practice (e.g. by using KLIK) is necessary to detect problems, offer the right help, and subsequently improve the wellbeing of children with CHCs.

Safety of a modified, low-protein infant formula in term infants: A Randomized, Double-blind, Equivalence Trial

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Introduction: A high protein intake in early life is associated with a risk of obesity later in life. The essential amino acid requirements of formula-fed infants have been reassessed recently, and the results enable a reduction in total protein content and thus in protein intake. Based on these outcomes a modified lower protein (mLP) infant formula was developed. The aim of this study was to assess the safety of this new infant formula in healthy, term-born infants. Outcomes were compared to standard protein (SP) infant formula.

Methods: In this double-blind randomized controlled equivalence trial, infants received either mLP (1.7 g protein/100 kcal; n=90) or SP formula (2.1 g protein/100 kcal; n=88) from enrollment (age ≤45 days) to 6 months of age. A breast-fed group served as a reference (n=67). Anthropometry and body composition were determined at baseline, 17 weeks, and 6 months of age. Urea and safety blood parameters were measured at the age of 17 weeks. The primary outcome was daily weight gain from enrollment up until the age of 17 weeks (at an equivalence margin of ±3.0 g/day) in the per protocol population.

Results: Weight gain (g/day) from baseline up until the age of 17 weeks was equivalent between the mLP and SP formula group (mLP 27.9 vs SP 28.8 g/day; difference -0.86 g/day [90%CI -2.36 – 0.63]). No differences in other growth parameters, nor in adverse events were observed. Urea was significantly lower in the mLP formula group versus the SP formula group (-0.73 mmol/L [95%CI -0.51 – -0.96] P <0.001).

Conclusion: An infant formula with a modified amino acid profile and a lower protein content is safe and results in similar growth to that of infants fed standard protein formula. This—in combination with a lower urea level—suggests that protein metabolism is more efficient in infants fed the modified low-protein formula.

Poster 27

Exergaming for people with dementia in day care centres: results of a randomized controlled trial

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Introduction: Everyone can benefit from physical activity. However, people living with dementia (PwD) experience several barriers to engage in physical activities, such as risk of falls and orientation issues. Exergaming ("physical exercise interactively combined with cognitive stimulation in a gaming environment") may help overcome these barriers. This study evaluates the (cost-)effectiveness of exergaming compared to regular activities in day-care centres (DC's) among PwD and informal caregivers (IC). Facilitators and barriers to implementation are also investigated.

Methods: A cluster Randomized Controlled Trial, with a process evaluation alongside. PwD and IC were interviewed at baseline, at 3 and 6 months. Primary outcomes were mobility and physical activities of PwD. Secondary outcomes for both PwD and IC were physical, cognitive, social and emotional functioning, and quality of life. Additionally for IC: subjective burden and positive care experiences. Mixed model analyses were conducted. The economic evaluation was done for the primary outcome measures. For the process evaluation, thematic analysis was performed on data from online surveys, qualitative interviews and focus groups.

Results: Twenty DC's (11 exp, 9 control) in the Netherlands and 112 dyads (PwD/IC; 73 exp, 39 control) participated in the study. Preliminary results show that exergaming was beneficial regarding memory and social behaviour of PwD and feelings of competence of IC. Cost-effectiveness analyses showed that on average exergaming was more effective and more expensive than usual care. Exergaming was highly appreciated by participants, family and staff. Facilitators for successful implementation were a.o. easy accessibility of the exergames, support from management and colleagues, and finances.

Conclusion: This study contributes to the evidence base of innovative exercise interventions for people living with dementia. Safe opportunities for enjoyable physical exercise and insight into its benefits will enable and motivate PwD to be more physically active. Guidelines are given to promote successful implementation of exergaming.

Poster 28

The development of a game to facilitate pediatric patient participation in hospital care, research and intervention development

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Introduction: Participation of patients in hospital care is essential, because patients can be seen as experts. Although this is increasingly acknowledged, professionals still find it hard to realize this, especially within pediatric patients. Therefore, the goal of this project is to develop a game for adolescents, called 'All Voices Count' ('Alle Stemmen Tellen'), which can be used by professionals to incorporate pediatric patient participation in hospital care, research and intervention development.

Methods: The game was developed in 3 steps; 1) focus groups with fifteen adolescents (age range 12-18y) with a chronic disease resulted in 10 major themes for adolescents regarding hospital care (e.g. my hospital and like me) and preferences for a group game that contains a winning element. 2) A first version of the game was developed based on the major themes. Fourteen adolescents (age range 12-18y) gave their opinion about the draft version. Overall, adolescents were positive about the game. It helps them to give their opinion more easily. However, the images on the cards should be more recognizable for adolescents. 3) Adjustments were made to the game. A pilot workshop with four adolescents (age range 13-16y) was held at the Emma Children's Hospital: the game is easy to play, but a word accompanying the image would be supportive.

Results: A final version of the game was developed, including a training for professionals, a game manual and a website (www.allestemmentellen.nl). All Voices Count can now be provided to researchers and health care professionals in the Netherlands.

Conclusion: With this game we provide professionals a tool to include the input from pediatric patients in the decision-making process of hospital care, research and intervention development.

Poster 29

Exploring the effect of Souvenaid on cerebral glucose metabolism in early Alzheimer's disease, the NL-ENIGMA trial

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Introduction: Alzheimer's disease (AD) is associated with synaptic loss. Souvenaid, containing multi-nutrient combination Fortasyn Connect, was designed to improve synapse formation. The NL-ENIGMA study explored the effect of Souvenaid on synaptic function in early AD by assessing cerebral glucose metabolism (CMRglc) with 18F-fluorodeoxyglucose ([18F]FDG) positron emission tomography (PET).

Methods: We conducted an exploratory double-blind randomised controlled single-center trial. Fifty patients with mild cognitive impairment or mild dementia with evidence of amyloid pathology (CSF or PET) were stratified for MMSE (20-24 and 25-30) and randomly 1:1 allocated to 24 weeks of daily administration of 125mL of Souvenaid (n=25) or placebo (n=25). [18F]FDG-PET scans with arterial sampling were acquired at baseline and 24 weeks. CMRglc was estimated by quantitative (Ki) and semi-quantitative measurements in five predefined regions of interest (ROI) and a composite ROI. Change from baseline CMRglc was compared, in the per-protocol population (PP), between treatment groups by ANOVA adjusted for baseline CMRglc and MMSE stratum. Additional exploratory outcome parameters were voxel-based analyses by Statistical parametric mapping (SPM).

Results: [18F]FDG-PET data was available for quantitative (placebo n=19, intervention n=18) and semi-quantitative (placebo n=20, intervention n=22) analyses. No baseline differences between treatment groups were found (PP, placebo/intervention: n=20/22; age 66.2yr±7.4/65.1yr±6.2; female 40%/45.5%; MMSE 25.1±2.9/24.6±3.1). No difference in change of CMRglc between treatment groups in any of the ROIs was found by semi- and quantitative analyses. No treatment effect was found in the semi-quantitative reference region by quantitative measures. The additional SPM analyses did not yield consistent differences between treatment groups.

Conclusion: We found no robust effect of Souvenaid on synaptic function measured by [18F]FDG-PET. Possible explanations include lack of power because of small group sizes and too short duration of treatment.

Poster 30

Anti-proliferative therapy with 6-mercaptopurine in Pulmonary Arterial Hypertension: *Hemodynamic improvement at the cost of serious side effects*

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Introduction: Pulmonary arterial hypertension (PAH) is a heterogeneous, incurable group of diseases characterized by exuberant proliferation of the pulmonary vascular endothelium ultimately leading to right heart failure. Current vasodilator therapies, although effective, do not target the proliferative cause of the disease. Preclinical data indicated that the clinically available drug 6-mercaptopurine (6-MP) mitigates endothelial cell proliferation *in vitro* and reverses pulmonary vascular remodeling *in vivo*. Hence, the aim of this clinical study was to evaluate the safety and efficacy of 6-MP as treatment for PAH.

Methods: Open label, single-center proof-of-concept study. Primary endpoint was reduction in pulmonary vascular resistance (PVR) as measured by right heart catheterization. Secondary endpoints include improvement in right ventricle function, exercise capacity, functional status and quality of life. Stable PAH patients, as determined by 6-minute walking distance, received 1.5mg/kg 6-MP orally once daily in addition to conventional vasodilatior therapy, for a period of 4 months.

Results: 77/184 PAH patients were eligible for inclusion and 15 patients were enrolled in the study. 75% of the approached patients declined to participate, predominantly because of fear of side effects and high study burden. 25% of included patients dropped out because of severe side effects, such as severe pancytopenia. We found a significant decrease in PVR (-120 dynes/sec/cm-5 p=0.004) after 6-MP treatment, however this did not lead to an increase in cardiac output, right ventricle function or functional status. Quality of life statements such as fatigue and feelings of depression were increased after treatment (p=0.009).

Conclusion: Anti-proliferative add-on therapy with 6-MP decreases PVR. Nevertheless, right ventricular function did not improve wherefore 6-MP treatment shows no clinical benefit. Frequency and severity of the side-effects lead to the conclusion that 6-MP is not a safe alternative as treatment for PAH patients. However, the decrease in PVR indicates that targeting proliferation might be of interest for future therapeutic approaches.

Poster 31

Sustained changes in body composition during 6 months follow-up after combined lifestyle intervention in older adults with obesity and type 2 diabetes

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Introduction: Patients with obesity and type 2 diabetes (T2D) are advised to reduce their body weight to lose fat mass. We recently showed that older adults with obesity and T2D who consumed a whey protein drink enriched with leucine and vitamin D lost fat mass and preserved muscle mass during a 3m combined lifestyle intervention of hypocaloric diet and resistance exercise (PROBE study). We now evaluated to what extent body composition change was sustained after 6m follow-up without intervention.

Methods: 105 older adults with obesity and T2D completed the 3m PROBE intervention and were followed from 3 to 9m after baseline. 76 subjects participated at 9m, of whom 38 had received the whey protein drink (test) and 38 had received an isocaloric control drink (control) during intervention. Body weight (scale), lean mass, appendicular muscle mass and fat mass (DXA), and dietary intake and physical activity level (3d record) were assessed. Change over time was analysed using paired samples t-test. ANOVA was used for evaluation of differences in change in body composition between test and control.

Results: At 9m, an average of 2.1 kg (78%) of the initial 2.7 kg weight reduction at 3m was maintained. Lean mass significantly increased (+0.60 ± 2.2 kg, p=0.026), whereas fat mass (+0.03 ± 2.8 kg, p=0.94) and appendicular muscle mass (+0.18 ± 0.98 kg, p=0.12) did not change. Protein intake (0.84 ± 0.32 g/kg body weight) returned to baseline level. The observed slight increase in physical activity level during 3m intervention (+0.05 ± 0.02, p=0.009) was maintained during 6m follow-up. There were no significant differences between the test and control group during follow-up.

Conclusion: Body weight change during the 3-month combined lifestyle intervention in the PROBE study was sustained after 6 months follow-up without intervention. In addition, lean mass had increased, whereas fat mass remained unchanged.

Poster 32

MR guided Radiotherapy in the clinic – the first completed study in prostate cancer

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Introduction: Since April 2016, magnetic resonance-guided radiation therapy (MRgRT) is clinically available in Amsterdam UMC, location VUmc. This technique is extremely suitable for stereotactic body radiation therapy (SBRT). MRgRT offers improved soft-tissue imaging, daily online adaptive planning and continuous imaging during treatment delivery allowing for the use of smaller uncertainty margins. As first institute in Europe using this technology in clinical practice, more than 550 patients have been treated for several tumor sites. More than 200 patients were treated for localized prostate cancer and here we focus on the first completed prospective MRgRT study in this patient group.

Methods: Within the trial, 101 patients with cT1-3bN0M0 prostate cancer were treated using stereotactic MRgRT in 5 fractions within two weeks. Gastrointestinal (GI) and genitourinary (GU) toxicity was studied using clinician reported outcomes (CRO) on pre-set study time-points at baseline, last fraction, after 1.5 and 3 months (CTCAEv.4). In addition, patient reported outcomes (PRO) were collected using quality of life questionnaires at the same time-points (EORTC QLQ C30, QLQ PR25 and IPSS).

Results: All patients completed MRgRT. The patients questionnaire response rate at 3 months was 100%. The maximum cumulative grade ≥2 early GI toxicity was 5% and this low incidence was confirmed by PRO data. No early grade 3 GI toxicity was observed. The maximum cumulative grade ≥2 early GU toxicity measured by any symptom at any study time point was 23.8%. GU grade ≥2 toxicity peaked to 19.8% at the end of MRgRT, followed by a return to the baseline average score at three months follow-up.

Conclusion: This innovative stereotactic MRgRT approach in patients with localized prostate cancer was well tolerated and showed a low incidence in both CRO and PRO measurements of particularly early GI, but also GU toxicity.

Poster 33

Work Ability and Vitality in Coach Drivers: An RCT to Study the Effectiveness of a Self-Management Intervention during the Peak Season

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Introduction: This RCT evaluates the effectiveness of a self-management toolbox designed to maintain work ability and vitality in coach drivers over their peak season.

Methods: The intervention group received a self-management intervention providing advice aimed at increasing work ability and vitality. These suggestions targeted three specific domains: work-recovery-rest balance, food and drink intake, and physical activity. At the beginning (March), middle (July), and end (October) of the coach sector peak season, work ability, vitality, work-related fatigue, psychosomatic health, sleep complaints, and perceived mental exertion of coach drivers were assessed through questionnaires.

Results: A total of 96 drivers participated in the study. Access to the toolbox did not result in significant differences between groups. Work ability and vitality decreased significantly in both groups, falling from 7.8 (±1.3) to 7.3 (±1.6) and from 63 (±16.7) to 55 (±18.7), respectively. Work-related fatigue increased from 35 (±31,9) to 52 (±35,3). Psychosomatic health complaints, sleep complaints and perceived mental exertion also increased significantly.

Conclusion: The uptake of the intervention was too low to determine if this toolbox can maintain work ability and vitality in coach drivers when compared with a control group. Overall work ability and vitality decrease significantly as the peak season progresses, while work-related fatigue accumulates. Other interventions should be explored to ensure sustainable employability in this population.

Indocyanine Green Fluorescence Angiography Guidance for Resection of Small Bowel Neuroendocrine Tumours

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Introduction: Radical resection of the primary tumour and mesenteric metastases of small bowel neuroendocrine tumours (SB-NETs) might compromise vascularization, Fluorescence angiography (FA) using indocyanine green (ICG) has been described as a safe technique to assess bowel perfusion during gastro-intestinal-surgery and aids the surgeon to decide on the optimal transection level. This study aims to correlate FA findings to patient outcomes after resection of SB-NETs.

Methods: This is a prospective proof-of-principle-study, currently still open for inclusion, conducted at a referral centre for SB-NETs. All patients undergoing surgery for SB-NET, of any stage, with continuity restoration and >18 years were eligible for inclusion. During surgery the planned transection level was marked by the surgeon, after which FA was performed using ICG(0.1 mg/kg/bolus) and the PINPOINT FA System (Stryker, Kalamazoo, U.S.A.). The primary study outcome is change in management due to FA. Secondary outcomes included postoperative hospital stay and the occurrence of anastomotic leakage within 90 days postoperatively.

Results: Currently, the cohort exists of four patients, of whom two male and two female. Three patients had a grade 1 SB-NET and one a grade 2 SB-NET, all with local lymph node metastases. The use of FA resulted in change of management in all cases: two patients had a tissue sparing resection of 15 and 35 cm, respectively, and extra tissue was resected in two patients of 8 and 13 cm, respectively. Median (IQR) hospital stay was 5(4-9) days. No anastomotic leakages were observed.

Conclusion: This is the first known series with preliminary results describing the use of FA during resection of SB-NETs. The use of FA led to change in management in all included cases, preventing unnecessary resection of healthy tissue or resection of poorly perfused small bowel. We expect to observe a decrease in post-operative complications related to small bowel perfusion in a later stage of the study.

Poster 35

Neuropathological correlates of parkinsonism in a large Netherlands Brain Bank autopsy series

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Introduction: Parkinsonism is a core feature of Parkinson's disease (PD) and several other neurological diseases, collectively termed atypical parkinsonisms. Here, we aimed to gain more insight in neuropathological correlates underlying clinical heterogeneity in PD and atypical parkinsonisms in a large Netherlands Brain Bank autopsy series.

Methods: We included 293 donors with a history of parkinsonism without dementia at disease onset, collected by the Netherlands Brain Bank (NBB) from 1989 to 2015. We compared the final clinical diagnosis of the treating physician with the neuropathological diagnosis. A subset of 234 donors with sufficient clinical information received a diagnosis based on retrospectively applied Movement Disorder Society (MDS) clinical diagnostic criteria for PD. National Institute on Aging – Alzheimer's Association guidelines were applied for pathological staging of alpha-synuclein, amyloid-β, neurofibrillary tau and neuritic plaques.

Results: Lewy pathology (LP) was present in 150 out of 176 donors (85%) with a clinical diagnosis of PD, and in 12 out of 117 donors (10%) with atypical parkinsonism. Independent from age at death, stages of amyloid-β, but not neurofibrillary tau or neuritic plaques, were higher in clinical PD ($p=0.009$) and donors with LP ($p=0.001$). A clinical diagnosis of PD and MDS criteria predicted the presence of LP with an accuracy of 88.0% and 90.6% respectively. In donors with LP, donors with and without atypical symptoms for PD according to MDS criteria showed similar stages for alpha-synuclein, amyloid-β, neurofibrillary tau and neuritic plaques.

Conclusion: In conclusion, both a clinical diagnosis of PD and MDS criteria for PD accurately predicted the presence of LP in NBB donors. In donors with LP, there was no neuropathological correlate of symptoms atypical for PD. A clinical diagnosis of PD was not only associated with LP, but also with presence of amyloid-β pathology, suggesting a link between amyloid-β accumulation and PD pathogenesis.

Poster 36

Long-term CIN3+ risk of HPV positive women after triage with methylation analysis

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Introduction: Primary high-risk human papillomavirus (hrHPV) testing in cervical screening is more sensitive for the detection of high-grade cervical intraepithelial neoplasia (CIN) and cervical cancer compared with cytology, and has therefore been implemented in several countries. However, since most HPV infections are transient and do not cause clinically relevant disease, additional triage tests are required to identify only HPV positive women who are at high risk of developing cervical cancer. This study evaluates the long-term risk for cervical intraepithelial neoplasia grade 3 or worse (CIN3+) among HPV positive women triaged with *FAM19A4/miR124-2* methylation analysis.

Methods: In a post hoc analysis, data on *FAM19A4/miR124-2* methylation, cytology, and HPV16/18 genotyping of HPV positive women (n=1 025) from a large population-based screening cohort with 14-year follow-up were evaluated. Cumulative CIN3+ incidences over 3 screening rounds (5-year intervals) of 4 triage strategies were compared: *FAM19A4/miR124-2* methylation analysis, cytology, HPV16/18 genotyping with *FAM19A4/miR124-2* methylation, and HPV16/18 genotyping with cytology.

Results: Kaplan-Meier estimates of 14-year cumulative CIN3+ incidence of HPV positive women with a negative methylation and a negative cytology triage test were comparable (16.3% and 15.6%, respectively). The cumulative CIN3+ incidence of methylation positive and cytology positive women were 39.8% and 46.5%, respectively. HPV16/18 genotyping with methylation and HPV16/18 genotyping with cytology resulted in the lowest 14-year cumulative CIN3+ incidence among triage negative women (10.7% and 10.0%, respectively), but cumulative CIN3+ incidence among triage positive women was lower (33.4% and 35.7%, respectively) compared with triage by methylation alone and cytology alone.

Conclusion: Among HPV positive women of 30 years and older, a negative *FAM19A4/miR124-2* methylation triage test provides a similar long-term CIN3+ risk compared with a negative cytology triage test. Because of their high CIN3+ risk, women with a positive methylation triage test could be referred for colposcopy. Therefore, *FAM19A4/miR124-2* methylation analysis is a promising alternative to cytology for triage of HPV positive women.

Poster 37

DNA methylation biomarkers in cervical cancer screening with self-sampling

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Introduction: The development of cervical cancer (CC) through well-defined precursor lesions (i.e., cervical intraepithelial neoplasia (CIN)) takes over 20-30 years, which offers opportunity for intervention and has contributed to the success of cervical screening. Over last decades, cytology-based screening has resulted in a reduction of the incidence of and mortality from CC. To further improve the effectiveness of cervical screening, high-risk (hr) HPV testing and self-sampling have been introduced in the Dutch screening program in 2017. HPV self-sampling is an effective method to reach screening non-attendees, and has recently shown to have similar clinical accuracy as primary HPV testing on physician-collected samples within an organized screening setting. To further improve this approach, a triage test directly applicable to self-sampled material is warranted to identify hrHPV-positive women at risk of CC. Candidate molecular disease markers for triage testing involve the host cell epigenetic changes, such as DNA hypermethylation, that following a transforming hrHPV infection drive progression to CC. DNA methylation markers have shown good performance to triage screening non-attendees with hrHPV-positive self-samples. In this project, we will clinically validate DNA methylation markers as a triage test that is directly applicable to self-samples among regular screening attendees.

Methods: Archived specimens from regular screening attendees participating in a randomized trial evaluating self-sampling in cervical screening will be used (Polman Lancet Oncol 2019). The paired screen positive design allows for evaluating paired HPV-positive self- and physician-collected samples. All samples will be subjected to quantitative methylation-specific PCR (qMSP) assays to assess methylation status of different DNA methylation markers using a multiplex approach, in which B-actin is used as a reference gene.

Results: The project will evaluate a series of candidate DNA methylation markers (n=8) for triage of hrHPV-positive screening samples. A total of 885 hrHPV-positive self-samples and 873 hrHPV-positive physician-collected samples are currently subjected to qMSP analysis. The results will be presented at the meeting.

Conclusion: Host cell DNA methylation analysis provides an attractive tool for direct triage on hrHPV-positive self-samples. Following clinical validation, a transition towards full molecular self-screening in routine hrHPV-based cervical screening programs can become feasible.

Poster 38

The discussion of uncertainty concerning multigene panel testing during cancer genetic counseling. An observational study.

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Introduction: Multigene panel testing is increasingly used to improve the identification of an inherited cancer predisposition. Pre-test counseling about multigene panel testing involves an increased level of uncertainty, for example about the meaning of identified variants. Ideally, counselees are fully informed about uncertainties to make an informed decision about whether or not to perform such a test. It is presently unknown whether and how uncertainty is discussed during initial cancer genetic counseling. We therefore investigated in which counselors discuss uncertainty concerning multigene panel tests, address counselees' uncertainties, and whether their manner is associated with counselors' characteristics.

Methods: Counsellors of all eight genetic centers in the Netherlands were instructed to discuss a multigene panel test with a SP. Using a script, SPs represented a counselee visiting for initial cancer genetic counselling who had had multiple cancer types. All consultations were videotaped and double-coded by two coders independently, using a coding scheme based on previous qualitative studies. Before and after the consultation, counselors completed a survey. Counselors' uncertainty expressions, initiative- and framing of expressions, and counselors' verbal responses to SP's scripted uncertainty expressions were coded.

Results: Counselors (N=29) expressed uncertainties mainly regarding scientific topics (94%) and on their own initiative (95%). Most expressions were framed directly (77%; e.g. We don't know), and non-valenced (59%; i.e. without a positive or negative value). After SPs expressed uncertainties, counselors mainly responded by explicitly referring to the uncertainty (69%) without providing space for further disclosure of the uncertainty (66%). More experience with genetic counseling led to a decrease in space for further disclosure of SPs' uncertainties ($p<.02$).

Conclusion: Mainly communicating scientific uncertainties, and using mostly space-reducing responses, raises the question whether enough attention is paid to counselees' personal uncertainties allowing them to disclose their concerns during initial genetic counseling.

Poster 39

Associations between nutrient intake and nutrient levels in blood in a memory clinic cohort; the NUDAD project

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Introduction: Dietary questionnaires and nutrient levels in blood provide estimates of micronutrient intake. The correlation between these two measures is however affected by e.g. reliability of self-reported intake and metabolic influences. This relation might be even more complex in a population with varying cognitive abilities. Such information is however important for the interpretation of nutrition research in memory clinic cohorts. We therefore aim to examine the relations between nutrient intake and their corresponding nutrient levels in blood in patients with mild cognitive impairment (MCI), Alzheimer's disease (AD) and controls.

Methods: We included 22 MCI (70±7 y, F 27%), 29 AD patients (70±9 y, F 52%) and 39 controls (63±7 y, F 54%), from the NUDAD project. Nutrient intake was assessed from a 238-item food frequency questionnaire, self-reported supplement use and blood nutrients. Nutrient intake/blood levels of vitamin A, B1, B6, B9 (folic acid), B12, C, zinc, total omega-3 fatty acids, DHA and EPA were included. Linear regression models examined associations of dietary and supplement intake (determinant) with nutrient levels (outcome).

Results: Supplement users had higher blood levels of B- and C vitamins, omega-3 fatty acids, DHA and EPA ($p<0.05$). Nutrient intake and nutrient levels in blood of B- and C vitamins, DHA and EPA were moderately associated in the total group ($\beta=0.2$-0.7, $p<0.05$). Stratification for diagnosis showed largely similar associations. This was confirmed by formal tests for interaction between nutrient intake and diagnosis that were not significant, except for vitamin C, DHA and EPA ($p<0.01$).

Conclusion: These results suggest that supplement use is an important predictor of nutrient blood levels. Moreover, nutrient intakes and blood nutrient levels measure related concepts for DHA, EPA, B- and C vitamins but less so for vitamin A, zinc and total omega-3 fatty acids. These associations seem largely similar across different diagnosis groups in a memory clinic cohort.

Poster 40

The 6-year longitudinal association of omega-3 polyunsaturated fatty acids with depressive disorders and their characteristics

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Introduction: Causality of the association of low omega-3 polyunsaturated fatty acid (n-3 PUFA) plasma levels with depression remains questionable. To determine the underlying nature of these associations, this study examined the bidirectional longitudinal associations of n-3 PUFA plasma levels with (presence, onset and course of) depressive disorders and symptoms.

Methods: Baseline (n=2912, 28.6% with current depressive disorder) and the 6-year follow-up data (n=1966, 13.0% with current depressive disorder) of the Netherlands Study of Depression and Anxiety (NESDA) was used. Depression diagnoses and symptoms were based on psychiatric interviews and self-report questionnaires. N-3 PUFA levels (ratio of total fatty acids (mmol%)), were assessed using nuclear magnetic resonance.

Results: Although n-3 PUFA levels at baseline were lower among depressed persons, linear and Cox regression analyses showed these levels were not consistently associated with subsequent change in depressive symptoms, onset or remission of depressive disorders over six years. Generalized Estimating Equations (GEE) showed that having a depressive disorder at baseline was associated with overall lower n-3 PUFA levels (main effect depression: Beta=-.204, SE=.047, p<.001) over 6 years, although these associations weakened over time (depression-by-time: p=.011). GEE showed that over six years, change in depressive disorders was not consistently accompanied by change in n-3 PUFA levels over time.

Conclusion: Despite significant cross-sectional associations between n-3 PUFA plasma levels and depression, this 6-year longitudinal study could not confirm a uni- or bidirectional association over time. The association between depression and n-3 PUFA plasma levels may not be due to a direct causal relationship.

Poster 41

Plasma amyloid beta 1-42 to 1-40 measured by novel Simoa Amyblood assays and glial fibrillary acidic protein predict Alzheimer's pathology

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Introduction: Alzheimer's pathological changes start decades before onset of clinical symptoms and can be identified using biomarkers. Preclinical Alzheimer's Disease (AD) might be the prime intervention window for disease. In this respect, plasma biomarkers for Alzheimer's Disease (AD) might help in identification of these clinical trial candidates and will help as well in therapeutic effectiveness monitoring. We aimed to validate the utility of plasma biomarkers Abeta1-42/1-40, glial fibrillary acidic protein (GFAP) and neurofilament light (NfL) for AD pathology as assessed with amyloid Positron Emission Tomography (PET).

Methods: 252 subjects from the Amsterdam Dementia Cohort with a baseline abnormal amyloid PET status (n=176: 63 ± 7 years, 87 females, 18 syndrome diagnosis subjective cognitive decline (SCD), 26 mild cognitive impairment (MCI), 132 AD), or normal amyloid PET status (n=76: 61 ± 9 years, 27 females, 52 SCD, 24 MCI) were included. Simoa assays were applied for Abeta1-42/1-40 (in-house developed (Amyblood, in collaboration with ADx neuroscience), GFAP and NfL (Quanterix kits) analysis. Associations between plasma levels and amyloid PET status were assessed using univariate analyses of variance and ROC analyses. An optimal prediction panel was identified using Wald's backward elimination logistic regression analysis.

Results: Adjusted for age and gender, plasma Abeta1-42/1-40 was decreased ($p<0.001$), and GFAP ($p<0.001$) and NfL ($p<0.001$) were increased in PET amyloid abnormal compared to amyloid normal. All plasma markers could predict an abnormal amyloid PET status (all: AUC>71%). When combining with AD risk factors, the optimal panel predicting amyloid PET included plasma Abeta1-42/1-40, GFAP, age and APOE (AUC=88% (95%CI:83–93%), 82% sensitivity, 86% specificity). Focusing on non-demented only (SCD+MCI), the optimal panel included plasma Abeta1-42/1-40, GFAP, and APOE (AUC=84% (95%CI:76–92%), 70% sensitivity, 86% specificity).

Conclusion: Plasma markers Abeta1-42/1-40 ratio and GFAP can be used to identify AD pathology.

Poster 42

Diagnostic value of plasma phosphorylated tau181 in Alzheimer's disease and frontotemporal lobar degeneration

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Introduction: Plasma phosphorylated tau181 (pTau) is a candidate diagnostic blood-based biomarker that differentiates Alzheimer's disease (AD) from other dementias. Its performance against frontotemporal lobar degeneration (FTLD) has not been tested. Objective: To examine whether plasma pTau differentiates between AD and FTLD, and how it relates to other AD biomarkers, including beta amyloid positron emission tomography (Aβ-PET) and tau (18FFlortaucipir) PET (FTP-PET), plasma neurofilament light chain (NfL), brain volume, and clinical status.

Methods: 309 participants were recruited. The cohort (248 UCSF, 19 ARTFL) consisted of 45 normal controls, 39 AD, 40 mild cognitive impairment (MCI) and 143 FTLD spectrum individuals, including 166 with Aβ-PET (92 negative, 74 positive), 75 with FTP-PET and 107 had autopsy-confirmed diagnoses or were autosomal dominant FTLD carriers.

Results: 135 (50.6%) female participants (67 ± 10 years). Plasma pTau concentrations were increased in AD and differentiated AD from other clinical diagnoses (AUC=0.904, 95%CI: 0.866-0.942, p<0.0001). pTau strongly correlated with cortical FTP-PET standardized uptake value ratio (β=0.73, p<0.0001), differentiated between Aβ-PET positive and negative participants, regardless of clinical diagnosis (AUC=0.920, 95% CI: 0.0.876-0.964, p<0.0001), and correlated with grey matter atrophy in AD and MCI participants. Plasma NfL was higher in FTLD than AD and controls and correlated with grey matter atrophy in frontotemporal regions, but not with amyloid PET or FTP-PET. Higher baseline pTau predicted faster functional and cognitive decline in AD and MCI, but not in controls or FTLD.

Conclusion: Plasma pTau is a sensitive biomarker of AD pathology. Plasma pTau and NfL measurements may be useful diagnostic biomarkers for neurodegenerative dementia that are less expensive and easier to deploy than PET.

Poster 43

Pulmonary hypertension incidence and patientcharacteristics in a nonreferral setting; results from the Dutch OPTICS registry

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Introduction: Until now, all epidemiologic data on PH is based on data from tertiary referral centers, over-representing cases of PAH and underrepresenting patients with PH due to left heart disease (PH-LHD). No epidemiologic data on the presence of PH and its subtypes in a nonreferral setting is currently available.
OBJECTIVES:
1.To determine the incidence and distribution of different types of PH in a nonreferral setting in the Netherlands.
2.To characterize patients at nonreferral centers with respect to demographics and comorbidities.

Methods: Clinical data of patients with pre- or post-capillary PH were obtained from a Dutch registry (OPTICS1) on incident PH patients from 13 nonreferral centers. Inclusion criteria were: Incident patients with an estimated Tricuspid Regurgitant Jet (TRJ) ≥ 2.8 ms-1 and/or other echocardiographic signs of pulmonary hypertension. Also patients in whom the presence and/or degree of PH is not explained by heart failure due to a reduced left ventricular ejection fraction or aortic or mitral valve disease. Multidisciplinary consultation for PH was warranted.

Results: A total 439 patients were enrolled in the OPTICS1 registry between January 2015 and July 2018. The mean age of the cohort was 71 ± 12 years and 41% was male. Within our non-referral cohort, the largest group (45%) consisted of post-capillary PH patients. PAH was seen in 10% of patients. However, within the cohort of patients referred to an expert clinic, the largest group (32%) consisted of PAH and post-capillary PH was seen in 23% of patients. The incidence of PH, other than PH due to systolic or valvular left heart disease, was estimated at 65 per million adults in the Netherlands.

Conclusion: In this unique cohort of patients, directly derived from nonreferral centers, post-capillary PH represents the largest group. The incidence of PH in the Netherlands was 65 per million adults.

Poster 44

Incorporation of differentiated dysplasia improves prediction of oral leukoplakia patients at increased risk of malignant progression

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Introduction: Oral leukoplakia is the most common oral potentially malignant disorder with a malignant transformation rate into oral squamous cell carcinoma of 3% annually. The presence and grade of World Health Organization defined dysplasia is an important histological marker to assess the risk for malignant transformation, but is not sufficiently accurate to personalize treatment and surveillance. Differentiated dysplasia, known from differentiated vulvar intraepithelial neoplasia, is hitherto not used in oral dysplasia grading. We hypothesized that assessing differentiated dysplasia besides World Health Organization defined (classic) dysplasia will improve risk assessment of malignant transformation of oral leukoplakia.

Methods: We investigated a retrospective cohort consisting of 84 oral leukoplakia patients. Formalin fixed, paraffin-embedded biopsy specimens were obtained and 3 µm sections were stained with hematoxylin-eosin to assess dysplasia presence and grade, and for two cytokeratins, keratin 13 (CK13) and keratin 17, known to be dysregulated in dysplastic vulvar mucosa.

Results: In dysplastic oral lesions, differentiated dysplasia is as common as classic dysplasia. In 24 out of 84 (29%) patients, squamous cell carcinoma of the upper-aerodigestive tract developed during follow-up. When classic and differentiated dysplasia are taken into consideration only 2 out of 30 (7%) patients with non-dysplastic lesions progressed. Risk of progression increased from 2.97 (Hazard ratio, $p = 0.005$) when only classic dysplasia is considered, to 7.02 (Hazard ratio, $p = 0.002$) when classic and differentiated dysplasia are combined. Loss of CK13, combined with presence of dysplasia, is associated with a greater risk of malignant progression ($p = 0.010$).

Conclusion: This study demonstrates that differentiated dysplasia should be recognized as a separate type of dysplasia in the oral mucosa and that its distinction from classic dysplasia is of pathological and clinical significance since it is a strong (co)prognostic histopathological marker for oral malignant transformation. In oral lesions without dysplasia and retained CK13 staining the risk for progression is very low.

How do undergraduate nursing students learn in the hospital setting? A scoping review of conceptualisations, operationalisations and learning activities

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Introduction: Despite its relevance to nursing education, there are gaps in our knowledge about clinical learning and the terminology to describe this. This study aimed to examine how concepts equivalent to "learning in practice" are used and operationalised and which learning activities are reported in the nursing education literature. The final aim was to propose terminology to guide future studies.

Methods: The scoping framework proposed by Arksey and O'Malley was used. Two systematic searches were conducted in PubMed, EBSCO/ERIC and EBSCO/CINAHL; first to identify concepts equivalent to 'learning in practice' and second to find studies operationalising these concepts. Eligible articles were studies that examined the regular learning of undergraduate nursing students in the hospital setting. Conceptualisations, theoretical frameworks and operationalisations were mapped descriptively. Results relating to how students learn were synthesised using thematic analysis. Quality assessment was performed using the Critical Appraisal Skills Programme (CASP) checklist.

Results: From 9360 abstracts, 17 articles were included. Five studies adopted a general, yet not explained, synonym for learning in practice, the other studies approached the topic focusing on the social, unplanned, or active nature of learning. All studies used a qualitative approach. The small number of studies and medium study quality hampered a thorough comparison of concepts. The synthesis of results revealed five types of learning activities, in which autonomy, interactions, and cognitive processing were central themes. These themes were acknowledged by an expert panel.

Conclusion: The current body of literature offers little guidance on the use of specific concepts to study clinical learning. Studies agree on the key elements of clinical learning. In future research, formal and informal components of learning should be addressed, and clarity about desirable outcomes of clinical learning should be provided. Also, the interplay between behaviour and cognitive processing should be further investigated.

Poster 46

Important topics for SOPs and guidelines for research integrity

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Introduction: Research integrity (RI) refers to conducting research according to high professional, ethical and methodological standards. To foster RI, it is necessary to not only optimize the behaviour of individual researchers, but also the organisational elements that influence researchers' behaviour. Therefore, the SOPs4RI project aims to develop a publicly available toolbox containing Standard Operating Procedures (SOPs) and guidelines for research performing organizations (RPOs) and research funding organizations (RFOs), targeted at the institutional level. To determine which topics should be covered in the toolbox, we are conducting two Delphi studies. Aim: Develop an expert-based consensus on which topics are important at the institutional level for optimizing and fostering RI in RPOs (study 1) and RFOs (study 2).

Methods: Based on expert knowledge and a literature search of topics mentioned in codes and guidelines in RPOs and RFOs across Europe, we developed an initial list of topics for each of the studies. We defined 'experts' as people with experience in research policy in RPOs or RFOs. Using a web search and SOPs4RI's network, we identified 305 and 220 experts in RPOs and RFOs, respectively. In the first round of each Delphi, we asked experts to rate the importance of each topic in our list on a 1-5 scale. Experts were also encouraged to provide comments for their ratings and to suggest new topics. Consensus was defined as 80% agreement on ratings 4-5.

Results: Based on the first round, there was consensus on including 8/14 topics from RPOs and 3/11 from RFOs.

Conclusion: We are currently preparing the second Delphi round, to explore consensus for 1) the remaining topics in our original list of topics and 2) newly proposed topics. After the second round, we will be informed by experts on which topics to include in SOPs4RI's toolbox.

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Interventions focusing on mental health or mental capacity of student and novice nurses to prevent dropout: a systematic review

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Introduction: Dropout among student and novice nurses is high. Mental health problems are a potential cause of dropout, and occur frequently. To prevent attrition due to reduced mental health/mental capacity, an overview of relevant interventions was needed. This systematic review provides 1) an overview of interventions aimed at improving mental health/mental capacity of student or novice nurses to prevent dropout from nursing education or nursing work and 2) an overview of the effectiveness of these interventions on dropout-related outcomes.

Methods: Up to 19 February 2019 various relevant scientific databases were searched. Two researchers identified studies. Interventions aiming at improving mental health with a quantitative research design were eligible for inclusion. Methodological quality was appraised.

Results: From 8,463 records, 21 studies met the inclusion criteria. Most studies focused on novice nurses (n=16); five studies targeted student nurses. Three types of interventions were identified: interventions aimed at: managing stress or stressors (n=4), facilitating the transition to nursing practice (n=14), and facilitating the transition to nursing practice with a stress management component (n=3). Designs varied from randomized controlled trials (RCT) to cross-sectional studies. Only one RCT was found. Five studies showed a statistically significant effect on one of the dropout-related outcomes: attrition, retention, sickness absence, and intention to stay. Among these five studies, two contained interventions aimed at managing stress or stressors targeting nurse students, two at facilitating the transition to nursing practice targeting novice nurses, and one intervention had a combined approach targeting novice nurses. The overall risk of bias was high.

Conclusion: A broad range of interventions aimed at improving mental health to prevent dropout from nursing education/work are available, but the evidence for the effectiveness of these interventions is limited. There is a need for high-quality studies in this field, preferably with a randomized controlled design.

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Enhanced Liver Fibrosis Test, a non-invasive diagnostic test for non-alcoholic fatty liver disease: A systematic review and Meta-analysis

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Introduction: There is an urgent need to develop non-invasive biomarkers for diagnosing non-alcoholic fatty liver disease (NAFLD), due to the limitations of liver biopsy as the reference standard. Recently, the Enhanced Liver Fibrosis (ELF) test has been suggested as a non-invasive serum biomarker to aid the diagnosis of advanced liver fibrosis in NAFLD patients. Objective: To provide a summary estimate of the accuracy of the ELF test against liver biopsy.

Methods: We searched Medline, EMbase, Web of Science and the Cochrane Library, for studies included NAFLD patients and undertook both liver biopsy as the reference standard test and the ELF test within a reasonable timeframe. Two authors independently screened the references, extracted the data and assessed the quality of each included study using the QUADAS-2 checklist. Due to the variation in thresholds reported by the studies we used a multiple thresholds, random effects model for meta-analysis (diagmeta R-package).

Results: We searched Medline, EMbase, Web of Science and the Cochrane Library, for studies included NAFLD patients and undertook both liver biopsy as the reference standard test and the ELF test within a reasonable timeframe. Two authors independently screened the references, extracted the data and assessed the quality of each included study using the QUADAS-2 checklist. Due to the variation in thresholds reported by the studies we used a multiple thresholds, random effects model for meta-analysis (diagmeta R-package).

Conclusion: The results of our meta-analysis showed that the ELF test can be considered as an accurate biomarker to diagnose advanced fibrosis just in very high prevalence settings. Clinicians should carefully consider disease prevalence in their practice setting and set the test threshold locally, to achieve the desired test performance in terms of PPV and NPV.

Co-creating care pathway management by stimulating reflectivity

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Introduction: Although Care Pathway Management aims to enhance the value for patients by restructuring care services, patients are barely involved in these processes. As those intended to benefit from care pathways, patients have relevant experiential knowledge on the provision of care. This study aims to acquire insights into how patients' involvement in co-creating CPM can be improved by stimulating reflectivity.

Methods: This study monitored and evaluated the implementation of patients' involvement in developing and managing the care pathways of Sarcoma and GIST at Erasmus Cancer Institute in the Netherlands, using the Interactive Learning and Action methodology. Qualitative data was collected via interviews, observations and interactive reflections. For analysis, the evaluation framework of Caron-Flinterman (2005) and the criteria for knowledge co-creation of Pittens et al. (2013) were used.

Results: Patients' involvement was found to be worthwhile for making specific improvements. The knowledge co-creation process encountered however repeated obstacles. Limited opportunities to overcome patients' knowledge gap and consult fellow sufferers hindered the articulation of knowledge. Existing power differences and limited time prevented the integration of knowledge. Knowledge embedment was endangered owing to uncertainties about responsibilities. Difficulties in overcoming these barriers were caused by limited willingness to reflect on the process due to time constraints, power balances and professionals' restricted role perceptions.

Conclusion: To enhance the value for patients, by involving them in co-creating Care Pathway Management, barriers of interactive reflections have to be overcome. A bottom-up approach, digital reflection methods and including reflective practices in professionals' education are suggested as possible solutions.

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The impact of physician-patient eye contact on patients' trust – exploring novel methods

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Introduction: Eye contact is a crucial aspect of non-verbal communication between patients and physicians. The use of Electronic Medical Records (EMR) may critically reduce eye contact. This is problematic, as reduced eye contact may hinder rapport building between patient and physician, indicated by a reduction in patients' trust levels. Our aim is to assess whether physicians' degree of eye contact predicts patient reported quality of the physician-patient relationship.

Methods: An observational study using mobile eye-tracking techniques, patient and physician surveys and video recording. Resident physicians in an Internal Medicine outpatient clinic wore eye tracking glasses while conducting follow-up consultations. Consultations were video recorded. Questionnaires were completed before and after the consultation by physicians and patients, assessing background characteristics, patients' personality traits, and outcomes such as trust. Artificially intelligent vision engineering techniques were used to analyse the level of eye contact.

Results: A total of 107 patients (50 female, mean age 58) consulting with 16 different physicians (8 female, mean age 34) participated. Preliminary analysis on 43 patients from 8 physicians revealed wide variation in the percentage of time physicians looked patients in the eye (range 14-71, m=36%). The level of eye contact during the first 30 seconds predicted looking behaviour throughout the consultation. Eye contact did not predict patients' trust in the physician.

Conclusion: This study was the first to use mobile eye tracking during medical consultations. We suggest that physicians have their personal looking style, since the first 30 seconds predicted the level of looking at the patient later in the consultation. The physicians' looking style could explain the large variance that we found in looking behaviour. Our preliminary results also advance evidence on the implication of EMR use for keeping eye contact with the patient and the consequences thereof for the quality of the relationship between patient and physician.