PARACELSUS
MEDICAL
UNIVERSITY

ABSTRACTBAND 2023

virtual Science Get Together
Paracelsus Science Summer

www.pmu.ac.at/forschung-innovation

Vorwort

Der alljährliche „Paracelsus Science Get Together“ konnte 2023 wieder in „altem Glanz“ durchgeführt werden – ergänzt um die virtuelle Plattform #vSGT, die aus Gründen der Notwendigkeit in der Pandemie eingeführt wurde, aber gekommen ist, um zu bleiben. Am 30.06.2023 konnten wir über 100 Besucher*innen, Forscher*innen und Interessierte beider Standorte sowie externe Gäste an der PMU in Salzburg begrüßen und gemeinsam einen Tag der Forschung feiern.

Forschende aus beiden Standorten der Universität, den Universitätskliniken in Salzburg und Nürnberg sowie Kooperationspartnern aus Salzburg und Nürnberg reichten insgesamt mehr als 100 internationale Poster (davon 9 Poster von Studierenden der Humanmedizin) ein. Sie stellten ihre aktuellen Forschungsarbeiten vor – virtuell und den ganzen Sommer über auch online verfügbar. Die gezeigten Arbeiten umfassen eine enorme thematische Bandbreite aus den verschiedensten Wissenschaftsbereichen, darunter Medizin, Natur- und Sozialwissenschaften, von Grundlagen- bis zu klinischer Forschung.

Rund 250 Besucher*innen haben online die medizinisch-wissenschaftliche Leistungsschau im bereits 13. Jahr ihres Bestehens in neuem Format besucht, wie auch die zahlreiche Teilnahme am eingeführten Publikumsvoting beweist. Mehr als 470 Seitenaufrufe wurden über den Sommer gezählt.

Herzliche Gratulation an alle Gewinner*innen der Poster Awards 👑

Die Fachjury entschied sich für folgende Preisträger*innen:

Lara Bieler mit “Exploring the potential of human iNSCs to restore connectivity in the injured spinal cord in a rat model” und **Vera Paar** mit “SGLT2 inhibitors reduce cytosolic calcium transient in a diabetic cardiomyopathy rat model with preserved ejection fraction” (Best Poster female, beide Salzburg). Das Best Poster male teilten sich **Bruno Benedetti** (Salzburg) mit “Old prince meets young sleeping beauty“: the slow awakening of dormant neuronal precursors in the aging brain” und **Lothar Marischen** (Nürnberg) mit “A case: 60-year old patient with refractory B cell lymphoma successfully treated with γδ T cells”. Das Best PhD Poster ging an **Michael Kleindorfer** (Salzburg) mit „Does early Montelukast therapy improve bladder function and spinal cord regeneration after SCI?”. **Theresa Mähr** (Salzburg) mit “The Cdk-5 inhibitor Dinaciclib as a novel approach in biliary tract cancer” und **Teresa Schotte** (Nürnberg) mit “IL-10 family cytokines in the interrelation of osteoarthritis and type 2 diabetes mellitus” wurden in der Kategorie Best Student Poster ausgezeichnet.

Alle Preisträger*innen stellten ihre Arbeit am 30. Juni in einem flotten Poster-Slam vor, der auch unter www.pmu.ac.at > Forschung & Innovation > Forschungsorganisation > Science Get Together online nachzuschauen ist.

Das Publikum kürte über den Science Summer hinweg das Poster von **Christiane Licht** (Nürnberg) mit dem Titel „Age effects on motor threshold“ zum besten Poster.

Ein herzlicher Dank gilt unseren Organisator*innen rund um Dorothea Kölblinger, der Leiterin des Forschungsmanagement Salzburg, sowie den zahlreichen Unterstützer*innen, die auch in diesem besonderen Jahr die Durchführung des vSGT mitermöglicht haben. Die vier internationalen Sponsoren Der Westerhof, Ipsen, Bayer und New England Biolabs, vor allem der Hauptsponsor Der Westerhof (Inhaber: Dr. Andreas Greither), der auch die Preise der diesjährigen Research and Innovation Preisträger*innen gesponsert hat, vertrauten auf das neue Format und ermöglichten das umfangreiche, internationale Netzwerken.

Wir freuen uns, Ihnen zum Abschluss des „Paracelsus virtual Science Get Together – Science Summer“ wie in jedem Jahr diesen Abstractband zum besseren Überblick und als Nachschlagewerk übermitteln zu dürfen, wiederum mit einer ISBN-Nummer versehen und damit zitierfähig.

Der besondere Dank der Universität gilt neben den Mitarbeiter*innen des Forschungs-management Salzburg und Forschungsmanagement und Services Nürnberg, der gesamten IT, die das neue Format bestens betreute. So wurde der Netzwerkgedanke des vSGT nicht nur von den Teilnehmenden, sondern auch von der Organisation gelebt – ganz im Sinne des Leitbildes der Universität.

Es ist uns ein großes Anliegen, den Vernetzungsgedanken weiter zu verankern und auch gemeinsame Forschungsprojekte zwischen den beiden Universitätsstandorten Salzburg und Nürnberg im Sinne eines Brückenschlages innerhalb der PMU weiter voranzutreiben und zu fördern.

Für 2024 laufen jetzt schon die Planungen: Es wird sowohl vor Ort als auch den ganzen Sommer über standortübergreifende virtuelle und hybride Veranstaltungen geben. Bleiben Sie gesund und neugierig und freuen Sie sich mit uns auf den „Science Get Together 2024“, bei dem wir wieder großartige Leistungen präsentieren werden.

Es grüßen Sie herzlich

Univ.-Prof. Dr. Wolfgang Sperl
Rektor

Univ.-Prof. Dr. Ludwig Aigner
Vizerektor für Forschungsangelegenheiten

Inhaltsverzeichnis

Der Paracelsus virtual Science Get Together 2023 wurde mit freundlicher Unterstützung folgender Unternehmen ermöglicht:

The place to ski
... do mog i hi!

BERGHOTEL SUDELFELD

Das Westerhof Hotel in Bayrischzell

Hotel & Alm mitten in der Natur

Tagen, Feiern, Wandern, MTB,
Wintersport am Sudelfeld

Unteres Sudelfeld 4 • 83735 Bayrischzell • Tel. (+49) 8023 81 99-0
info@berghotel-sudelfeld.de • www.berghotel-sudelfeld.de

Der Westerhof

TEGERNSEE · HOTEL

The place to be
... do mog i hi!

Dahoam in Traumlage

Zimmer / Suiten mit einmaligem Seeblick
Inmitten der schönsten Wanderwege

Olaf-Gulbransson-Straße 19 • 83684 Tegernsee
Tel. (+49) 08022 - 188 988 • www.derwesterhof.de

… ÜBER DIE ERKRANKUNG

Überlegene Wirksamkeit gegenüber Sunitinib mit einem **OS-Vorteil von 14 Monaten** (49,5 vs. 35,5 Monate).*[,2]

… ÜBER DIE BEHANDLUNG

Mit flexibler Dosierung und einem vertrauten Verträglichkeitsprofil.[3]

… ÜBER DIE PATIENTENERFAHRUNG

Mit durchgängig überlegenen HRQoL-Vorteilen gegenüber Sunitinib.[4]

CABOMETYX® ist als Monotherapie bei fortgeschrittenem Nierenzellkarzinom (RCC) indiziert für die Erstlinienbehandlung von erwachsenen Patienten mit mittlerem oder hohem Risiko sowie bei Erwachsenen nach vorangegangener zielgerichteter Therapie gegen VEGF (vaskulärer endothelialer Wachstumsfaktor).[3,5–7] CABOMETYX® ist als Kombinationstherapie mit Nivolumab für die Erstlinienbehandlung des fortgeschrittenen Nierenzellkarzinoms bei Erwachsenen indiziert.[2,3,8,9]

*HR: 0,70 (95% KI: 0,56–0,87; p = 0,0014); stratifizierter LogRank-Test, p-Werte waren deskriptiv. Für die HR wurde die stratifizierte Cox-Regression verwendet.

HR: hazard ratio; **HRQoL:** gesundheitsbezogene Lebensqualität (health-related quality of life); **KI:** Konfidenzintervall; **OS:** Gesamtüberleben (overall survival).

1. Leitlinienprogramm Onkologie (Deutsche Krebsgesellschaft, Deutsche Krebshilfe, AWMF): S3-Leitlinie Diagnostik, Therapie und Nachsorge des Nierenzellkarzinoms, Langversion 4.0, Februar 2023. **2.** Burotto M et al. Presented at CITIM Conference; April 24–27, 2023; Vilnius, Lithuania. **3.** Aktuelle Fachinformation CABOMETYX®. **4.** Cella D et al. J Clin Oncol. 2022;40(suppl 6):323. **5.** Choueiri TK et al. Lancet Oncol. 2016;17(7):917–927. **6.** Choueiri TK et al. Eur J Cancer. 2018;94:115–125. **7.** Motzer RJ et al. Br J Cancer. 2018;118(9):1176–1178. **8.** Choueiri TK et al. N Engl J Med. 2021;384(9):829–841. **9.** Motzer RJ et al. Lancet Oncol. 2022;23(7):888–898.

Fachinformation

www.ipsen.com/germany/produkte/

CABOMETYX® (cabozantinib) Tabletten + NIVOLUMAB

IPSEN Innovation for patient care

CBZ-DE-001482 05/2023

NEW ENGLAND BioLabs® GmbH

be INSPIRED
drive DISCOVERY
stay GENUINE

Extreme Fidelity in PCR.

Q5® High-Fidelity DNA Polymerase

NEBs Q5 High-Fidelity DNA Polymerase setzt den Industriestandard in der PCR und verbindet extreme Genauigkeit (>280× genauer als *Taq*) mit höchster Performance!

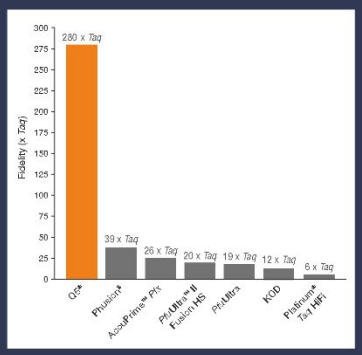
Auch auf unterschiedlichsten Templates (bis 20 kb, AT-reich, GC-reich) ist die Q5 besonders zuverlässig und daher unter Forscher*innen sehr beliebt – sogar im Weltall auf der ISS. Dank kurzer Elongationszeiten und PCR-Protokolle sparen Sie mit der Q5 Polymerase wertvolle Laborzeit.

Alle Produktdetails, die Vorteile für Ihre Forschung sowie ein kostenfreies Testmuster* erhalten Sie unter:

www.neb-online.de/Q5

* Solange der Vorrat reicht. Angebot ist begrenzt.

NEBs Q5 HiFi DNA Polymerase bietet Ihnen „fehlerfreie" und zuverlässige PCR Amplifikationen – zum fairen Preis.



Q5 Polymerase ist >280 mal genauer als Taq DNA Polymerase.
Warum sollten Sie sich mit weniger zufrieden geben?

Die Mandarinente (Aix galericulata) ist in der chinesischen Kunst ein gebräuchliches Symbol für Treue und Genauigkeit (engl. „fidelity").

Abstractsammlung

Cdk5 inhibtion as novel approach to enhance the efficacy of Lenvatinib in thyroid cancer

Celina Ablinger[1], Raffael Höllerer[1], Petra Huber-Cantonati[1], Johanna Pachmayr[1]

[1]Institute of Pharmacy, Department of Pharmaceutical Biology and Clinical Pharmacy, Paracelsus Medical University, Strubergasse 15, 5020 Salzburg; Contact: celina.ablinger@pmu.ac.at

Objective

Thyroid cancer is the eighth most frequently cancer worldwide with increasing incidence. Thyroidectomy is the cornerstone of therapy for differentiated thyroid cancer (DTC), followed by administration of radioactive iodine (RAI), which results in good prognosis. However, resistance against RAI often leads to disease recurrence. In this context, the multi-kinase inhibitor Lenvatinib was approved for refractory-DTC (RR-DTC) treatment. Since the therapy is accomplished by severe side effects and/or resistance development, there is a substantial need for novel treatment options (1). In this context, Dinaciclib, a specific cyclin-dependent kinase 5 (Cdk5) inhibitor, was shown to induce apoptosis, cell cycle arrest and inhibits tumor growth in multiple types of cancers including thyroid carcinoma (2). The aim of the underlying study is thus to investigate the potential synergistic effect of Lenvatinib and Dinaciclib combination treatment in the DTC cell line K1.

Methods

To assess the proliferation ability of the DTC cell line K1, cells were treated with different concentration of Dinaciclib and Lenvatinib alone or in combination for 72 h. Migration rates of K1 cells after treatment with Dinaciclib/Lenvatinib or combination were investigated with culture inserts from Ibidi®, whereas inserts illustrate a wound via an open gap. Migratory cells were continuously measured with a Tecan Spark plate reader over 24 h. To determine changes in the expression levels of proliferation and migration related genes after Dinaciclib/Lenvatinib or combination treatment, real-time PCR analyses were performed. For protein quantification a standard Western Blot procedure using proliferation (AKT) and migration (Vimetin) markers was conducted. Band intensities were quantified with the ImageLab software from Bio-Rad.

Results

The combination treatment of Lenvatinib and Dinaciclib significantly reduces cell proliferation in K1 cells in a synergistic manner. K1 cells are able to migrate after Lenvatinib and Dinaciclib treatment. In contrast, the migration ability of double-treated cells is significantly decreased. The RNA levels of the analysed genes Cdk5, TGFB, FGFR, AKT, Vimentin and EGFR are less expressed after combination treatment. Moreover, Dinaciclib and the combination treatment significantly decreases protein levels of AKT in contrast to Lenvatinib treatment.

Conclusions

The Cdk5 inhibitor Dinaciclib enhances the efficacy of Lenvatinib treatment in K1 cells. The synergistic effect of Dinaciclib and Lenvatinib was displayed in different cell based assays. Nevertheless, additional experiments regarding molecular mode of action, cell death and in vivo experiments need to be done to improve future therapy options for RR-DTC patients.

Acknowledgements

This project is supported by the PMU-FFF research fund (R-19/03/120 ARD).

References

1. 1. Fallahi P, Ferrari SM, Galdiero MR, Varricchi G, Elia G, Ragusa F, Paparo SR, Benvenga S, Antonelli A. Molecular targets of tyrosine kinase inhibitors in thyroid cancer. Semin Cancer Biol. 2022 Feb;79:180-196. doi: 10.1016/j.semcancer.2020.11.013. Epub 2020 Nov 26. PMID: 33249201.
2. 2. Lin SF, Lin JD, Hsueh C, Chou TC, Wong RJ. A cyclin-dependent kinase inhibitor, dinaciclib in preclinical treatment models of thyroid cancer. PLoS One. 2017 Feb 16;12(2):e0172315. doi: 10.1371/journal.pone.0172315. PMID: 28207834; PMCID: PMC5312924.

OMEGA-3 FATTY ACID EPA SUPPLEMENTATION RESTORES GUT MICROBIOTA BALANCE IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

Barbara Altendorfer[1,2], Heike Mrowetz[1,2], Ariane Benedetti[2,3], Alina Bretl[1,2], Diana Marisa Bessa de Sousa[1,2], Sabine Bernegger[1,2], Anja Maria Ladek[4], Andreas Koller[4], Andrea Trost[4], Ludwig Aigner[1,2]

[1]Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg, Austria; [2]Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University, Salzburg, Austria; [3]Institute of Experimental Neuroregeneration, Paracelsus Medical University, Salzburg, Austria; [4]Research Program for Experimental Ophthalmology and Glaucoma Research, Department of Ophthalmology and Optometry, University Hospital of the Paracelsus Medical University, Salzburg, Austria; Contact: barbara.altendorfer@pmu.ac.at

Objective
Neuroinflammation is a major hallmark of Alzheimer's disease (AD) enhanced by inflammatory stimuli of a disturbed balance in the gut microbiota via the gut-brain axis. Dietary polyunsaturated fatty acids (PUFAs) have a high potential to modulate neuroinflammation and the gut microbiota. The omega-3 PUFA eicosapentaenoic acid (EPA) is metabolized much faster than docosahexaenoic acid (DHA) to lipid mediators with anti-inflammatory effects. Moreover, EPA competes for the same enzymes as arachidonic acid (AA). Thus, promoting EPA metabolism may inhibit the formation of pro-inflammatory omega-6 eicosanoids derived from AA. In this study, we investigate if supplementation with a high dose of EPA and a high EPA/DHA ratio (10:1) reduces neuroinflammation in the central nervous system and attenuates gut dysbiosis in the APP-PS1 mouse model.

Methods
Female APP-PS1 mice (RRID: MMRRC_034829-JAX) and non-transgenic littermates (WT), 13-14 months old, were fed a diet supplemented with 0.3% EPA or control chow for 3 weeks. The hippocampus and blood plasma was used for quantification of eicosanoids. RNA gene expression analysis of inflammatory markers was performed on retinal tissue. Fecal pellets were analyzed for gut microbiota composition.

Results
APP-PS1 mice had higher hippocampal levels of proinflammatory eicosanoids (e.g. 5-HETE, PGD2) than WT mice. Unexpectedly, WT animals had higher blood levels of 5-HETE. Supplementation with EPA had no effect on brain eicosanoids, but it significantly reduced 5-HETE blood levels in WT mice. H2Aa, the alpha chain of major histocompatibility complex class II, is commonly used as a marker of neuroinflammation and microglial activation. EPA diet reduced retinal expression of H2Aa at the mRNA level in APP-PS1 and WT mice, suggesting reduced microglia-driven inflammation. Microbiome analysis revealed elevated abundance of Bacteroidetes in the APP-PS1 mice, indicating genotype specific gut microbiota dysbiosis. EPA supplementation decreased the percentage of Bacteroidetes and increased bacteria of the phyla Firmicutes in APP-PS1 and WT mice. The ratio of Firmicutes to Bacteroidetes, which is known to decline in ageing and AD [1], was significantly increased by EPA-diet.

Conclusions
Short-term EPA supplementation counteracted gut microbiota dysbiosis in the APP-PS1 Alzheimer's mouse model and decreased expression of the pro-inflammatory microglial marker H2Aa in the retina. This short-term treatment had no significant effects on hippocampal eicosanoid levels. Prolonged dietary intervention could potentially extend the promising effects of gut microbiota on brain parenchyma.

References
1. Mariat, D et al. "The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age." BMC microbiology vol. 9 123. 9 Jun. 2009, doi:10.1186/1471-2180-9-123

Ketogenic diet enhances the anti-tumor effect of mifepristone in a chemical-induced breast cancer mouse model

Sepideh Aminzadeh-Gohari[1,2,3], Julia Tevini[1], Daniela D. Weber[1], Luca Catalano[1], Victoria Stefan[1], Elisa Redl[2,3], Twana Alkasalias[4], Chiara Herzog[2,3], Roland Lang[5], Thomas K. Felder[6], Barbara Kofler[1], Martin Widschwendtner[2,3,4,7]

[1]Research Program for Receptor Biochemistry and Tumor Metabolism, Dept. of Pediatrics, PMU, Salzburg, Austria; [2]European Translational Oncology Prevention and Screening (EUTOPS) Institute, University Innsbruck, Innsbruck, Austria, Hall in Tirol, Austria; [3]Institute for Biomedical Aging Research, University Innsbruck, Innsbruck, Austria; [4]Dept. of Women's and Children's Health, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden; [5]Dept. of Dermatology and Allergology, PMU, Salzburg, Austria; [6]Dept.of Laboratory Medicine, PMU, Salzburg, Austria; [7]Dept. of Women's Cancer, University College London, London, UK; Contact: j.tevini@salk.at

Objective

Progesterone and estrogen are known drivers of breast cancer formation and the hormone receptor status in breast cancer patients forms the basis for any personalized therapy (1). The aim of the study was to elucidate a potential enhancement of the already known anti-tumor effect of mifepristone (MIF) -a progesterone receptor antagonist- in combination with a low carbohydrate/high fat ketogenic diet in an immune-competent breast cancer mouse model.

Methods

Female BALB/c mice were fed either a control (CTRL) or a ketogenic diet (KD, ketogenic ratio 4:1) at 6 weeks of age. After one-week, mammary tumors were induced by subcutaneous implantation of 50 mg slow-release MPA (release for 90 days), followed by oral administration of 1 mg DMBA once weekly for a total of five to six times. On the same day of MPA implantation, mice also received 3 mg slow-release MIF pellet (release for 90 days) or placebo. Mouse body weight, blood parameters (glucose and β-hydroxybutyrate), tumor incidence, and tumor growth were monitored over the study period. Furthermore, we established a targeted HPLC-MS/MS approach to track the ultimate distribution of MIF and one of its active metabolites, metapristone (MET), in plasma and organs of interest.

Results

MIF effectively delayed breast tumorigenesis, resulting in a significant prolongation of both tumor-free and overall survival. The combination of MIF with the KD enhanced the anti-tumor effect of MIF, such that the greatest prolongation of tumor-free and overall survival was observed in the KD+MIF group. The KD alone also significantly delayed tumor occurrence compared to mice fed with the CTRL diet, although it failed to significantly slow tumor growth and subsequently to increase the overall survival. As expected, the KD induced ketosis in mice while blood glucose levels remained unaffected. MIF pellet implantation led to its metabolization into MET and in a systemic distribution in the organs with highest MIF levels in breast followed by uterus, breast tumor tissue and liver whereas spleen showed the lowest levels.

Conclusions

Our results show that KD is able to enhance the anti-tumor effect of MIF. Targeted HPLC-MS/MS measurements indicate an accumulation of MIF and its active metabolite MET at the desired side of action, namely breast tissue.

Acknowledgements

Supported by the ERC under the European Union's Horizon 2020 research and innovation program (no. 742432; BRCA-ERC), FWF (P31228-B33) and PMU (A-18/02/032-KOF, 2022-PRE-006-Catalano).

References

1. Osborne CK. Steroid hormone receptors in breast cancer management. 1998. PMID: 10068081.

A comparison between patients with ascending aorta replacement either through partial upper sternotomy or median full sternotomy

Matthias Angerer[1], Wolfgang Hitzl[2], Theodor Fischlein[1], Francesco Pollari[1]

[1]Cardiac surgery, Cardiovascular Center, Paracelsus Medical University-Klinikum Nuremberg, Nuremberg, Germany; [2]Research and Innovation Management (RIM), Team Biostatistics and Publication of Clinical Trial Studies, Paracelsus Medical University, Salzburg, Austria; Contact: matthias.angerer@stud.pmu.ac.at

Objective
The aneurysm of the ascending aorta poses a great danger for patients, even in times of modern medicine. Despite the danger, or precisely because of this, it is necessary to develop and establish new techniques for special operations such as ascending aorta replacement in order to reduce the surgical risk and risks for complications to decrease. [1]
While other cardiac surgical interventions, for example aortic valve replacement, are now increasingly being performed via minimally invasive approaches, [2] the replacement of the ascending aorta is lagging behind in this respect. Therefore, further studies are necessary to evolve the ascending aortic replacement.

The aim, this thesis is pursuing, is to show if there is a difference in relation to mortality and severe complications after surgical intervention between patients, who received an ascending aortic aneurysm repair via a partial superior ministernotomy (Figure 1 C) in comparison to patients, who were treated with the same operation through a standard full sternotomy (Figure 1 A).

Methods
A total of 167 patients were included into the study. After 1:3 propensity score matching along the variables of age, gender, BMI, EuroSCORE II and an additional aortic valve operation, 38 patients in the partial sternotomy group and 118 in the full sternotomy group were compared.

Results
Significant differences were observed in the rate of additional aortic root replacement with one patient (3%) in the PS and 22 patients (19%) in the FS group. Also variables of surgical time (from skin to skin) with means of 270 min (SD±63.4) and 218.9 min (SD±53.6), cross clamp time with means of 94.2 min (SD±26.6) and 83 min (SD±26.1), cardiopulmonary bypass time with means of 164.2 min (SD±47.2) and 126.8 min (SD±37.1) and ventilation time with means of 41.5 hours (SD±98.8) and 22.5 hours (SD±58.5) showed significant deviations with prolonged times in the PS group. Nevertheless no significant discrepancies were observed between the groups with regard to the postoperative and follow up complication reoperation and mortality rates. The non-significance regarding the mortality rates are shown in figure two measured with Kaplan Meier analysis.

Conclusions
Surgical treatment of an ascending aorta aneurysm via a minimally invasive partial upper sternotomy shows equal results to a median full sternotomy in terms of postoperative outcome and complication reoperation and mortality rates.

References
1. Wittenstein , G. Complications of Aortic Aneurysm Surgery: Prevention and Treatment. The Thoracic and Cardiovascular Surgeon, (1987) doi:10.1055/s 2007 1020275
2. Paparella D, Malvindi PG, Santarpino G, Moscarelli M, Guida P, Fattouch K et al. Full sternotomy and minimal access approaches for surgical aortic valve replacement : a multicentre propensity matched study . Eur J Cardiothorac Surg 2020;57:709 16.
3. Busch F. Entwicklung eines Simulators für den minimal invasiven operativen Aortenklappenersatz: Anforderungsanalyse und Evaluierung der Validität. Published online November 20, 2018. https://ul.qucosa.de/api/qucosa%3A32259/attachment/ATT 0/

Control system of a physiological relevant biomechanical test setup for the investigation of complex pelvic ring fractures

Dirk Baumeister[1,2], Jonas Mock[1,3], Markus Greinwald[1], Martin Winkler[1], Peter Augat[1,2]

[1]Institute for Biomechanics, BG Unfallklinik Murnau, Germany; [2]Institute for Biomechanics, Paracelsus Medical University, Salzburg, Austria; [3]Kempten University of Applied Sciences, Germany; Contact: dirk.baumeister@bgu-murnau.de

Objective

Fractures of the human pelvis can lead to disruptions of the ring-shaped structure which results in mechanical destabilization of the pelvic ring. A variety of different methods for an osteosynthetic treatment are available, but there is still a lack of knowledge about the mechanical stability of these methods. The aim of this project is to build a test setup able to determine the stability of different fixation methods for complex pelvic ring fractures under a loading situation simulating human gait.

Methods

During gait, the human pelvis is loaded mainly by the weight of the trunk and muscle forces. A control system is required to apply these forces to a pelvis for biomechanical testing. This control system must be able to adjust the required forces of the different actuators and to prevent undesired forces.

Results

The vertical force of the trunk is applied on the sacrum via a hydraulic testing machine. Hip joint endoprostheses are mounted to the acetabuli of the specimen. The vertical movement of the hip joint endoprostheses can either be released (swing leg phase) or locked (stance leg phase). The muscle forces are realized by two muscle pulls per side. The stepper motors programmed with Matlab/Simulink control the muscle pulls in such a way that the required forces can be achieved and undesirable situations do not occur in the test sequence.

Conclusions

To date, biomechanical experiments on pelvises have been performed primarily on single-leg stance or double-leg stance models. Because these do not fully take into account the complex loading situation during gait, the new test setup was developed. Simplifications were made to account for the applied loads, especially the muscle forces. The new test setup can be used to evaluate the fatigue strength of osteosynthetic constructs used for complex pelvic ring fractures.

Inducing ferroptosis, a new approach to target biliary tract cancer?

Dino Bekric[1], Daniel Neureiter[2], Heidemarie Dobias[1], Marlena Beyreis[1], Martina Winklmayr[6], Markus Ritter[1,3,4,6], Tobias Kiesslich[1,5], Christian Mayr[1,5]

[1]Center of Physiology, Pathophysiology and Biophysics, Institute of Physiology and Pathophysiology, Paracelsus Medical University, 5020 Salzburg, Austria; [2]Institute of Pathology, Paracelsus Medical University / Salzburger Landeskliniken (SALK), 5020 Salzburg, Austria; [3]Kathmandu Medical School of Medical Sciences, Dhulikhel 45200, Nepal; [4]Gastein Research Institute, Paracelsus Medical University, 5020 Salzburg, Austria; [5]Department of Internal Medicine I, Paracelsus Medical University/Salzburger Landeskliniken (SALK), 5020 Salzburg, Austria; [6]Ludwig Boltzmann Institute for Arthritis and Rehabilitation, Paracelsus Medical University, 50202 Salzburg, Austria; Contact: dino.bekric@pmu.ac.at

Objective

In 2012, Dixon et al. described a new form of regulated cell death, called ferroptosis. Ferroptosis is defined as an iron- and ROS-dependent non-apoptotic form of regulated cell death that leads to excessive peroxidation of polyunsaturated fatty acids (PUFAs), which ultimately results in cell death (1). Biliary tract cancer (BTC) is a deadly malignancy, with a poor overall survival rate, which lacks efficient therapeutic options. Therefore, the aim of this study is to our knowledge, a first-time investigation of ferroptosis as an anti-BTC strategy.

Methods

Resazurin assay and half maximal inhibitory concentration (IC_{50}) calculation were used to evaluate the anti-tumor effect of ferroptosis in a BTC in vitro model, using six different potential ferroptosis inducing substances (FINs; RSL-3, IKE; $FINO_2$, FIN56, iFSP1, Brequinar).
Rescue experiments using established ferroptosis, necroptosis and apoptosis inhibitors were performed to evaluate potential ferroptosis induction by these substances.
To further determine ferroptotic cell death, lipid ROS and intracellular iron were measured on a Spark multimode reader.

Results

The first results indicate a relatively heterogenous susceptibility of BTC cells towards ferroptosis inducing substances and morphological changes can be seen after a short period of time for selected cell lines. Cell death can be induced relatively early and with a low IC_{50} value range from 0.9 µM – 5.5 µM for selected FINs in KKU-055, CCC5 and HuH-28 cells, whereas signs of resistance towards ferroptosis induced cell death can be seen in OCUG and OZ. Cell death, induced by ferroptosis inducer, can be fully rescued by ferroptosis inhibitor deferoxamine, if HUH-28 cells were treated with $FINO_2$. Lipid ROS levels are doubled in CCC5 cells if treated with iFSP-1. In HUH-28 cells, intracellular ferrous iron levels are more than doubled, if treated with brequinar.

Conclusions

In this study, we can show in a first step, that cell lines react differently on cell viability and morphological levels, after treatment with ferroptosis-inducing substances. Furthermore, combination of ferroptosis-inducers with inhibitors of ferroptosis, necroptosis or apoptosis indicate ferroptotic events in the BTC cell line HUH-28. Interestingly, lipid ROS as well as intracellular Fe^{2+} levels are elevated in a cell-line and substance specific manner, after treatment with FINs. These results indicate, that ferroptosis can be induced in BTC and might therefore display a promising novel therapeutic strategy.

Acknowledgements

This project is funded by PMU-FFF research fund: E-20/31/164-MAC and is part of my PhD-Thesis: Medical Science MSI 2020

References

1. (1) Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell. 2012;149(5):1060-72.

"Old prince meets young sleeping beauty": the slow awakening of dormant neuronal precursors in the aging brain

Bruno Benedetti[1,2,3], Maximilian Reisinger[1,2,3], Marie-Theres Hochwartner[1,2,3], Gabriele Gabriele[1,2], Dominika Jakubecova[1,2,3], Ariane Benedetti[1,2,3], Thomas Weiger[4], Sebastien Couillard-Despres[1,2,3]

[1]Institute of Experimental Neuroregeneration, Paracelsus Medical University; Salzburg, Austria; [2]Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS); [3]Austrian Cluster for Tissue Regeneration, Vienna, Austria; [4]Department of Biosciences, University of Salzburg, Salzburg, Austria; Contact: bruno.benedetti@pmu.ac.at

Objective
Dormant neuronal precursors are peculiar cells of the adult mammalian brain (1). Their latent post-mitotic immaturity from birth to adulthood is followed by staggered awakening events over the entire course of life, according to mechanisms that are still largely unresolved. With this work, we aimed to determine whether elderliness hinders the maturation of dormant precursors. Thereby we tracked the maturation of these cells in the aging brain.

Methods
In transgenic mice (DCX-CreRT2/Flox-EGFP) the administration of tamoxifen allows to label immature dormant precursors permanently and to trace the course of maturation thereafter, throughout adulthood and elderliness. In this model, the maturation of dormant precursors was analysed in the adult and aging brain with immunohistochemistry and single-cell electrophysiology.

Results
Late-maturing precursors become fully developed neurons. However, aging and age of maturation onset affect the morphological traits of adult-matured neurons, including size of the soma, dendritic branching, length of the axon initial segment, and degree of synaptic connectivity. In regard to functional properties, adult matured neurons broadly resemble neonatal matured neurons of the same brain area. However, late maturation onset is associated with increased neuronal excitability, and with a striking reduction in spontaneous excitatory synaptic input.

Conclusions
Our work reveals that brain aging does not prevent the maturation of dormant precursors, but their morphological and functional traits depend on the age of maturation onset. Since latecomer neurons are more excitable, but less functionally integrated, controlling the age and speed of maturation may help to fine-tuning the integration and function of these cells in the adult brain networks.

Acknowledgements
This project was possible thanks to the generous support of the Paracelsus Medical University, Salzburg, PMU-RIF: 2021-SEED-003-Benedetti

References
1. Benedetti, B.; Couillard-Despres, S. Why Would the Brain Need Dormant Neuronal Precursors? Front. Neurosci. 2022, Apr 8, 16:877167, doi:10.3389/fnins.2022.877167

The dimerization of the anion exchanger pendrin

Emanuele Bernardinelli[1], Florian Huber[1], Silvia Dossena[1]

[1]Institute of Pharmacology and Toxicology, Paracelsus Medical University, Salzburg, Austria; Contact: e.bernardinelli@pmu.ac.at

Objective

The anion exchanger pendrin (SLC26A4, PDS) is expressed in the inner ear, thyroid and kidney. Defects in this transporter are associated with syndromic or non-syndromic sensorineural hearing loss. Pendred syndrome is characterized by hearing loss and iodide organification defects in the thyroid gland, eventually leading to goiter. The pendrin protein is predicted to have 14 transmembrane segments, with intracellular N- and C-termini. As other members of the SLC26 family are known to form dimers, here we investigate the possible existence of pendrin dimers and we identify a potential dimerization region.

Methods

Western Blot: lysates from cells overexpressing HIS-tagged-PDS were prepared using buffers with increasing denaturing strength. Proteins were separated by SDS-PAGE, transferred on PVDF and blotted with the appropriate antibodies.
Co-IP: lysates from cells overexpressing HIS-tagged and FLAG-tagged PDS were incubated with magnetic beads conjugated with an anti-HIS or an anti-FLAG antibody. Beads were washed and bound proteins were eluted. Samples were separated by SDS-PAGE, transferred to PVDF and blotted with the appropriate antibodies.
FRET: dimerization of pendrin was assessed by FRET in the acceptor photobleaching configuration. The FRET donor was PDS-ECFP, the FRET acceptor was PDS-EYFP.
Functional test: the influx of iodide was measured in cells co-expressing wild type and truncated forms of pendrin and an iodide-sensitive EYFP. Fluorescence was measured before and after injection of iodide in the bath solution. Decrease in fluorescence correlates with pendrin activity.

Results

Western blot showed the monomeric and dimeric forms of pendrin in different denaturing conditions. The dimeric form was favored in non-denaturing conditions, but still present in a strong denaturing buffer, indicating the formation of a very stable dimer.
Pull down experiments on lysates from cells expressing a FLAG-tagged-PDS and an HIS-tagged-PDS confirmed the existence of a FLAG-PDS/PDS-HIS heterodimer by reciprocal co-precipitation.
By FRET we showed that deletion of the transmembrane segment 14 resulted in loss of dimer formation with the wild type, while a longer truncation including the transmembrane 14 was still able to dimerize. Accordingly, the longer truncation gave a dominant negative effect on the function of the wild type.

Conclusions

In heterologous expression systems, human pendrin forms stable dimers. The dimerization sequence probably resides within the 14th transmembrane segment. Further investigations are necessary in order to characterize the dimerization interface and the pathophysiological significance of dimer formation.

References

1. Geertsma, E. R. et al. (2015). Structure of a prokaryotic fumarate transporter reveals the architecture of the SLC26 family. Nature structural & molecular biology, 22(10), 803–808. https://doi.org/10.1038/nsmb.3091
2. Alper SL, Sharma AK. The SLC26 gene family of anion transporters and channels. Mol Aspects Med. 2013 Apr-Jun;34(2-3):494-515. doi: 10.1016/j.mam.2012.07.009.

Clinical and demographic factors affecting trough levels of isavuconazole in critically ill patients with or without COVID-19

Ralph Bertram[1], Hans-Theodor Naumann[1], Vanessa Bartsch[2], Wolfgang Hitzl[3,4,5], Martina Kinzig[6], Golo-Sung Haarmeyer[7], Matthias Baumgärtel[7], Arnim Geise[7], Dorothea Muschner[7], Jens Nentwich[8], Stefan John[8], Fritz Sörgel[6,9], Joerg Steinmann[1], Rainer Höhl[1]

[1]Institute of Clinical Hygiene, Medical Microbiology and Infectiology, Klinikum Nürnberg, Paracelsus Medical University, Nuremberg, Germany; [2]Study Program in Human Medicine, Paracelsus Medical University, Nuremberg, Germany; [3]Department of Research and Innovation Management, biostatistics and publication of clinical trial studies, Paracelsus Medical University, Salzburg, Austria; [4]Department of Ophthalmology and Optometry, Paracelsus Medical University, Salzburg, Austria; [5]Research Program Experimental Ophthalmology and Glaucoma Research, Paracelsus Medical University, Salzburg, Austria; [6]Institute for Biomedical and Pharmaceutical Research, Nuremberg-Heroldsberg, Germany; [7]Department for Respiratory Medicine, Klinikum Nürnberg, Paracelsus Medical University, Nuremberg, Germany; [8]Department of Cardiology, Intensive Care Medicine, Klinikum Nürnberg, Paracelsus Medical University, Nuremberg, Germany; [9]Institute of Pharmacology, West German Heart and Vascular Centre, University of Duisburg-Essen, Essen, Germany; Contact: ralph.bertram@klinikum-nuernberg.de

Objective
The broad-spectrum antifungal isavuconazole is administered to treat invasive aspergillosis and mucormycosis. Isavuconazole plasma concentrations of critically ill ICU patients with or without COVID-19 and invasive fungal infection were determined and factors for subtherapeutic drug levels (<1µg/mL) were evaluated.

Methods
Isavuconazole plasma levels were measured as part of therapeutic drug monitoring (TDM) in ICUs of a tertiary hospital. Concentrations determined 20-28h after previous dosing were defined as trough (C_{min}) levels. A total of 160 C_{min} levels of 62 patients with invasive fungal infection were analysed, 30 of which suffering from COVID-19. Patient characteristics included into univariable and multivariable analysis were gender, age, COVID-19 status, body-mass index (BMI), sepsis-related organ failure (SOFA) score, renal replacement therapy (RRT) and extracorporeal membrane oxygenation (ECMO) requirement.

Results
The mean Cmin of isavuconazole in all patients was 1.64 µg/mL (interquartile range 0.83-2.24 µg/mL, total range 0.24-5.67 µg/mL). In total, 34.4% of the C_{min} values (corresponding to 46.8% of patients) were below a threshold concentration of 1 µg/mL. Drug concentrations between patients with or without COVID-19 did not differ (p=0.43). In contrast, levels were significantly lower in patients with female sex (p=0.0007), age≤65 years (p=0.002), BMI>25 (p=0.006), SOFA score>12 (p=0.026), RRT (p=0.017), and ECMO requirement (p=0.001).

Conclusions
Isavuconazole plasma levels can be negatively affected by patients' risk factors, supportive renal replacement and ECMO therapy. Future prospective studies analysing the relevance of isavuconazole drug levels in ICU patient outcome are urgently needed.

A case: 60-year old patient with refractory B cell lymphoma successfully treated with γδ T cells

Anna Bold[1], Lothar Marischen[1], Johannes Gärtner[1], Alexander Bott[1], Volker Mordstein[2], Kerstin Schäfer-Eckart[1], Stefan Knop[1], Martin Wilhelm[1]

[1]Department of Hematology and Medical Oncology, Paracelsus Medical University, Nuremberg, Germany; [2]Department of Pathology, Paracelsus Medical University, Nuremberg, Germany; Contact: lothar.marischen@klinikum-nuernberg.de

Objective
γδ T cells, which represent about 5% of T cells in human peripheral blood, offer strong cytotoxic effects on cancer cells *in vitro* and *in vivo* (1-3). Because of their major histocompatibility complex (MHC)-independent recognition of target cells, they can be safely used in an allogeneic setting (4). We have already demonstrated that the monoclonal antibodies rituximab and obinutuzumab (both anti-CD20) improve the cytotoxicity of γδ T cells against B cell malignancies *in vitro* (2). However, clinical studies regarding the combined use of γδ T cells and monoclonal antibodies are still missing. Here we present a patient with chemorefractory B cell lymphoma who was treated with haploidentical γδ T cells combined with obinutuzumab.

Methods
The patient received conditioning therapy with peripheral blood leukocytes from his daughter combined with cyclophosphamide and fludarabine. All-trans retinoic acid (ATRA) was given to sensitize the tumor cells and obinutuzumab was administered to enhance the cytotoxicity of γδ T cells. For the administration of γδ T cells, αβ T cells and B cells were depleted from the leukapheresis product from his daughter and the remaining cells were administered (5).

Results
γδ T cell therapy combined with obinutuzumab induced complete remission, which was histologically confirmed and lasted four months.

Conclusions
Combining a cellular therapy with γδ T cells with the CD20 monoclonal antibody is a promising approach for the therapy of B cell malignancies. The case reported here demonstrates the *in vivo* efficiency of this therapy also in chemorefractory situation. However, the patient relapsed after four months. Repeated administrations of the cells are probably necessary for this to persist in the long term. Therefore, the combination of *ex vivo* stimulated cells with monoclonal antibodies should be tested in clinical trials.

Acknowledgements
Anna Bold and this study were supported by “Verein Hilfe für Krebskranke e.V.” Nürnberg.

References
1. Morita CT, Mariuzza RA, Brenner MB. Antigen recognition by human gamma delta T cells: pattern recognition by the adaptive immune system. Springer Semin Immunopathol 2000; 22:191-217.
2. Hoeres T, Pretscher D, Holzmann E, Smetak M, Birkmann J, Triebel J, Bertsch T, Wilhelm M; Improving Immunotherapy Against B-Cell Malignancies Using γδ T-Cell-specific Stimulation and Therapeutic Monoclonal Antibodies. J Immunother. 2019 Nov/Dec; 42(9): 331-344.
3. Silva-Santos B, Serre K, Norell H. γδ T cells in cancer. Nat Rev Immunol 2015; 15:683-91.
4. Wilhelm M, Smetak M, Schaefer-Eckart K, Kimmel B, Birkmann J, Einsele H, Kunzmann V. Successful adoptive transfer and in vivo expansion of haploidentical γδ T cells. J Transl Med 2014; 12:45.
5. Bold A, Gaertner J, Bott A, Mordstein V, Schaefer-Eckart K, Wilhelm M. Haploidentical γδ T Cells Induce Complete Remission in Chemorefractory B-cell Non-Hodgkin Lymphoma. J Immunother. 2023 Feb-Mar 01;46(2):56-58.

The role of monoamines for the physiological modulation of microglia

Moritz Brosch[1,2,3], Bruno Benedetti[1,2], Ariane Benedetti[1,2], Lara Bieler[1,2], Tarik Col[1,2], Günter Lepperdinger[3], Sebastien Couillard-Despres[1,2]

[1]Institute of Experimental Neuroregeneration, Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University, Salzburg, Austria; [2]Austrian Cluster of Tissue Regeneration, Vienna, Austria; [3]Department of Biosciences, University of Salzburg, Salzburg, Austria; Contact: moritz.brosch@pmu.ac.at

Objective

Microglia are the resident immune cells of the central nervous system (CNS). They sense and respond to inflammation, but they also mediate physiological functions such as the refinement of synaptic plasticity and the support of adult neurogenesis. Microglia are readily alerted of injury and cell damage by molecules such as ATP or lipopolysaccharides (LPS) (1). The latter leads to a pro-inflammatory state, triggering not only the release of cytokines, but also a switch in pattern of receptor expression, which can result in different microglial sensitivity to several neuromodulatory molecules, including monoamines. Therefore, we questioned modulatory impacts of monoamines on resting and activated microglia.

Methods

We used murine immortalized microglia (BV-2) to perform calcium imaging, motility/migration assays, phagocytosis assays, immunohistochemistry, flow cytometry, gene expression analysis (RT-PCR). Microglia activation was readily achieved by acute or chronic stimulation with LPS and acute stimulation with ATP. Our preliminary analysis focused on the impact of monoaminergic modulation by serotonin (5-HT) and noradrenaline (NE) of microglia.

Results

Preliminary data suggest significant differences in the sensitivity of microglia to monoaminergic modulation between physiological and pathological conditions. For instance, NE triggered an acute intracellular calcium response in resting BV-2 microglia in a dose-dependent manner. However, responses to NE were potentiated when microglia were pre-activated by LPS. Conversely, no calcium responses were elicited following 5-HT stimulations of either resting or activated microglia. Moreover, chronic exposure to NE (but not 5-HT) desensitized both resting and activated microglia to further acute noradrenergic stimulation. In all conditions, calcium responses were readily elicited in microglia upon acute ATP application. With these data and further preliminary work, we shed new light on the complex and subtle balance between levels of monoamines and microglia responsiveness under pro-inflammatory states.

Conclusions

In the course of some pathologies, as well as in aging, the monoaminergic neuromodulation of the CNS changes in synchrony with altered microglial pro-inflammatory states. Thus, direct (neuron mediated) and indirect (microglia-mediated) consequences of altered monoaminergic neurotransmission on neuronal function, connectivity, and plasticity will need to be further scrutinized.

References

1. Pannell, M., Szulzewsky, F., Matyash, V., Wolf, S. A., & Kettenmann, H. (2014). The subpopulation of microglia sensitive to neurotransmitters/neurohormones is modulated by stimulation with LPS, interferon-γ, and IL-4. GLIA, 62(5), 667–679. https://doi.org/10.1002/glia.22633

Distribution of cysteinyl leukotriene system components in the human, rat and mouse eye

Susanne M. Brunner[1], Falk Schrödl[2], Julia Preishuber-Pflügl[1], Christian Runge[1,3], Andreas Koller[1], Anja-Maria Ladek[1], Markus Lenzhofer[4], Herbert A. Reitsamer[1,4], Andrea Trost[1]

[1]Research Program for Experimental Ophthalmology, Department of Ophthalmology and Optometry, University Hospital of the Paracelsus Medical University, Salzburg, Austria; [2]Center for Anatomy and Cell Biology, Institute of Anatomy and Cell Biology, Paracelsus Medical University, Salzburg, Austria; [3]Cornea eye bank, Department of Ophthalmology and Optometry, University Hospital of the Paracelsus Medical University Salzburg, Salzburg, Austria; [4]Department of Ophthalmology and Optometry, University Hospital of the Paracelsus Medical University Salzburg, Salzburg, Austria; Contact: su.brunner@salk.at

Objective

The cysteinyl leukotrienes (CysLTs) have important functions in the regulation of inflammation and cellular stress (1). Blocking the CysLT receptors (CysLTRs) is associated with a reduced development of retinopathies (e.g. diabetic retinopathy, wet age-related macular degeneration). However, the exact cellular localization of the CysLTRs and their endogenous ligands in the eye has not been clearly elucidated yet. It is also not known whether the expression patterns differ between human, rat and mouse eyes. Therefore, the present study aimed to describe and compare the distribution of 5-lipoxygenase (5-LOX) and 5-lipoxygenase-activating protein (FLAP), two important enzymes in CysLT biosynthesis, and of CysLTR1 and CysLTR2 in healthy human, rat and mouse eyes.

Methods

Human donor eyes (n=10) and eyes from adult Sprague Dawley rats (n=5) and CD1 mice (n=8) of both sexes were collected. The eyes were fixed in 4% paraformaldehyde and cross-sections were labeled by immunofluorescence with specific antibodies against 5-LOX, FLAP (human tissue only), CysLTR1 and CysLTR2. Flat-mounts of the human choroid were prepared and processed similarly. Expression patterns were evaluated using a confocal fluorescence microscope (LSM710, Zeiss).

Results

We observed novel, so far unreported expression sites for CysLT system components in various ocular tissues. Overall, we detected expression of 5-LOX, CysLTR1 and CysLTR2 in the human, rat and mouse cornea, conjunctiva, iris, lens, ciliary body, retina and choroid. Importantly, expression profiles of CysLTR1 and CysLTR2 were highly similar between human and rodent eyes. FLAP was expressed in all human ocular tissues except the lens. Largely weak immunoreactivity of FLAP and 5-LOX was observed in a few cells of diverse ocular tissues, indicating low levels of CysLT biosynthesis in healthy eyes. CysLTR1 was predominantly detected in ocular epithelia, supporting the involvement of CysLTR1 in stress and immune responses. CysLTR2 was predominantly expressed in ocular neurons and nerve fibers, suggesting neuromodulatory roles of CysLTR2 in ocular tissues and revealing disparate functions of CysLTRs in the eye.

Conclusions

Taken together, we provide a comprehensive protein expression atlas of CysLT system components in the human and rodent eye. While the current study is purely descriptive and therefore does not allow significant functional conclusions yet, it represents an important basis for future studies in diseased ocular tissues in which distribution patterns or expression levels of the CysLT system might be altered. In general, the elucidation of CysLT expression patterns in the eye helps to identify and understand functions of the system as well as mechanisms of action of potential CysLTR ligands in the eye.

References

1. Bäck M et al. Leukotriene receptor nomenclature, distribution, and pathophysiological functions. Pharmacol Rev 2011;63(3):539-84. PMID:21771892

Do systemic adverse events following mRNA vaccine BNT162b2 of a vaccinated hospital staff sample differ from the approval study sample?

Bunila Yuwang Francisca[1], Cosima Brucker[1], Annette Sattler[2], Isabel Hach[3], Wolfgang Hitzl[4]

[1] Clinic for Gynecology and Obstetrics, Paracelsus Medical Private University, Klinikum Nürnberg Nuremberg, Germany; [2] Hospital Pharmacy, Paracelsus Medical Private University, Klinikum Nürnberg, Nuremberg, Germany; [3] Education and Science Paracelsus Medical Private University Nuremberg, Klinikum Nürnberg, Germany; [4] Department Research and Innovaton Management (RIM) Biostatistics and publication of clinical trial studies, Paracelsus Medical University Salzburg, Salzburg, Austria; Contact: francisca.bunila@stud.pmu.ac.at

Objective
Our study is a COVID-19 vaccine pharmacovigilance study that evaluates age and gender differences of adverse events (AEs) in a hospital staff sample as well as differences between this sample and the approval study sample (1). It was designed to identify, assess, understand, prevent and communicate adverse effects of COVID-19 vaccines. Such studies are extremely important and help vaccinators avoid problems and protect people's health from adverse events during vaccination (2).

Methods
Employees of the Nuremberg hospital vaccinated with BNT162b2 participated in our study and provided anonymized information on health status (e.g., previous and actual illnesses) and adverse events following the first and second COVID-19 vaccinations within one week after the vaccination. We used standardized questionnaires designed as the adverse event reporting in the pivotal study to record local and systemic adverse drug reactions. Additionally we asked sleep disturbances and nervousness. We evaluated only the BNT162b2 vaccinated employees. Statistical analysis was carried out anonymously using methods of descriptive analysis and procedures on sample comparisons.

Results
First dose BNT162b2: (N=574) consists of 447 females (77.9%, 18-55years: N=342; >56 years: N= 105) and 127males (22.1%, 18-55 years: N=89; >56 years: N= 38). The most frequent systemic AEs of the approval study “fatigue”, “myalgia” and “headaches” occurred significantly more frequent in younger participants of our sample ($p<0.01$), and more often in 18-55 years old females than in males (i.e., female/male ratio: AE headache: 3.1; AE fatigue: 4.1; AE myalgia: 3:1). 18-55 year-old hospital workers showed significantly more often myalgia after the first vaccine dose than >56 years old participants of our sample and as well as the 16-55 year old participants of the approval study sample. In age group ≥56years of our sample, only women reported the AEs vomiting, chills, and diarrhoea.
Second BNT162b2 Dose: (N=347) consists of 283 females (81.6%, 18-55 years: N=196; >56 years: N=87) and 63 males (18.4%, 18-55 years: N=37; >56 years: N=26). The most frequent AEs were fatigue, headaches (same as for AEs after the first BNT162b2 dose), myalgia, and arthralgia. Headache was predominantly reported in the approval study sample in the age group >55yrs and in our sample, age group 18-55years predominantly reported headaches more in females (62.8%) than in males (51.4%).
AEs occurred rarely in the approval study sample placebo groups.

Conclusions
Our results suggest the need to report (vaccine) AEs in approval studies by gender and age group. For example, to investigate reasons for the differences, to find out the presence and the role of nocebo and placebo effects.

References
1. Polack FP et al. Safety and Efficacy of BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med 2020; 383:2603-2615 DOI: 10.1056/NEJMoa2034577
2. Kuçuku M. Role of pharmacovigilance on vaccines control*. J Rural Med. 2012;7(1):42-5. DOI: 10.2185/jrm.7.42. Epub 2012 Jul 7. PMID: 25648188; PMCID: PMC4309327.

Health status of health care workers – starting points for prevention?

Francisca Bunila Yuwang[1], Cosima Brucker[1], Wolfgang Hitzl[2], Annette Sattler[3], Isabel Hach[4]

[1]Gynecology and Obstetrics, Paracelsus Medical University, Nuremberg, Germany; [2]Research office (biostatistics), Paracelsus Medical University, Salzburg, Austria; [3]Hospital pharmacy, Paracelsus Medical University, Nuremberg, Germany; [4]Education and Science, Paracelsus Medical University, Nuremberg, Germany; Contact: isabel.hach@klinikum-nuernberg.de

Objective
Working in hospital is demanding on a psychological and physical level, not only in pandemic times. In Germany, employees in nursing showed an average of 22.4 days of absence from work due to sickness in 2020 (i.e. 8 days longer than other German employees, main causes: musculoskeletal and mental health problems). Health care workers are predominantly female (hospital staff: about 75% are females). There are different factors that are related to sick leave of hospital workers, e.g. high workload, adverse working conditions, poor physical health, burnout. The goal of our study was to determine the gender specific health status of health care workers after the first year of the Covid-19 pandemic.

Methods
Each employee of the Nuremberg hospital vaccinated with Covid-19 vaccines was offered the opportunity to participate in our study and to provide anonymized information on health status (e.g., previous and actual illnesses, body measurements). This study is part of a pharmacovigilance study about Covid-19 vaccines. We used standardized questionnaires (Paul-Ehrlich-Institute for Serious Adverse Event Reporting) and added two questions ("Do you worry about the Covid 19 vaccination process", "Do you feel well informed about the vaccine?"). The study was approved by the local institutional review board (IRB 2022_003). Statistical analysis was carried out anonymously using methods of descriptive analysis and procedures on sample comparisons.

Results
Our sample consists of 774 participants, 600 females (78%, 18-35years: N=153, 36-50 years: N=208, >51years: N=239) and 174 males (22%, 18-35years: N=51, 36-50 years: N=51, >51years: N=72). 25% of the female employees and 20% of male employees were smokers, the highest percentage of smokers was found in the group aged >51 years. Young female hospital workers were more often smokers than young male hospital workers (23.5% vs. 11.8%, $p<0.05$). 21% of female hospital staff >51y fulfilled criteria for obesity (BMI >30kg/m²). Suffering from allergies was the most frequently mentioned disease (females N=319, 53%, males N=88, 50%), prevalence rates increased with age, i.e. 69% of women >51years suffered from allergies. Cardiovascular (CV, particularly hypertension) and thyroid diseases (TH, particularly hypothyroidism) were frequent: (females CV: 11.5%, TH: 11.5%, males CV: 12% TH: 2.9%). Females were significantly more often affected from thyroid diseases than males ($p<0.001$). Muscle and joint problems were reported rarely, namely 5.3% of women (10% of >51years old females vs. males: N=2). 27.5% of women worried about the vaccination process, but only 10% of male hospital workers ($p<0.0001$).

Conclusions
Our sample shows several relevant public health problems and diseases (i.e. obesity, smoking cessation, hypothyroidism, associated diseases). We found sex and age related differences. Targeted prevention measures (behavioural and structural prevention) should start as soon as possible and should include the working environment.

References
1. Schug C, Geiser F, Hiebel N, Beschoner P, Jerg-Bretzke L, Albus C, Weidner K, Morawa E, Erim Y. Sick Leave and Intention to quit the Job among nursing staff in German Hospitals during the COVID-19 Pandemic. Int J Environ Res Public Health. 2022 Feb 10;19(4):1947. doi: 10.3390/ijerph19041947. PMID: 35206136; PMCID: PMC8872054.

The Development of Turn-Taking Between Infants and Fathers

Antonia Dinzinger[1,2], Selina Ismair[1,2], Beate Priewasser[1,2], Gabriela Markova[1,2,3]

[1]Institute for Early Life Care, Paracelsus Medical University, Salzburg, Austria; [2]Department of Pediatrics, University Hospital Salzburg, Paracelsus Medical University, Salzburg, Austria; [3]Faculty of Psychology, University of Vienna, Austria; Contact: antonia.dinzinger@pmu.ac.at

Objective
Shortly after birth infants begin to participate in closely coordinated interactions with their caregivers, synchronizing visual and auditory information in a complex flow of turn-taking (1), which has favorable consequences particularly for early communicative development (2). Although mothers and fathers interact differently with their children in several domains and, therefore, may affect child development in distinct ways, almost all studies investigated turn-taking with mothers only. It remains unexplored how turn-taking between fathers and their young infants develops and which paternal characteristics influence their children's communication skills. Consequently, the aim of the present study was to examine the development.

Methods
We observed naturalistic interactions between fathers and their infants (N = 40) at 8 weeks and 6 months post-partum. We micro-coded vocalizations of fathers and infants (excluding crying) and, subsequently, computed turn-taking sequences as paternal vocalizations followed by infant vocalizations, and vice versa, within 300ms (3). Additionally, we assessed fathers' ability for reflective functioning as well as the time they spent with their infants after birth.

Results
Preliminary analyses showed that infants at 8 weeks involved on average 39% of their vocalizations in turn-taking, whereas at 6 months this increased to 60%. The frequency of turn-taking increased significantly with age, both for turns taken up by the infants ($p = 0.050$) and turns taken up by their fathers ($p = 0.005$). When fathers had better reflective functioning, the overall increase in turn-taking between 8 weeks and 6 months was larger ($p = 0.029$). The frequency of infants overlapping paternal vocalizations or fathers overlapping infant vocalizations did not change with age, nor did paternal reflective functioning affect the overlaps. We found no effect of the amount of time fathers spent with their infants.

Conclusions
Results of the present study show that fathers and infants are increasingly involved in turn-taking sequences during early communicative exchanges, corroborating findings from studies examining mother-infant interactions. Our findings further indicate that fathers' reflective functioning favorably impacts this development. Since turn-taking requires that both communicative partners closely attend to each other and are sensitive to the other's communicative signals (3), reflective functioning may facilitate the quick and correct predictions of the other's vocal behavior. Our findings provide a more holistic picture of children's conversational development by suggesting that early father-infant interactions add to the wealth and complexity of communicative input children are exposed to.

References
1. Gratier, M, Devouche, E, Guellai, B, Infanti, R, Yilmaz, E, & Parlato-Oliveira, E. Early development of turn-taking in vocal interaction between mothers and infants. Front. Psychol. 2015 Sept 4, 6: 1167.
2. Levinson, S. Turn-Taking in human communication - Origins and implications for language processing. Trends in Cognitive Sciences. 2016 Dec 23, 20: 6-14.
3. Nguyen, T, Zimmer, L, & Hoehl, S. Your turn, my turn. Neural synchrony in mother-infant protoconversation. Phil. Trans. R. Son. B. 2023 March 6, 378.

VIRTUAL JAK-POT: IDENTIFICATION OF NEW JANUSKINASE INHIBITORS USING AN IN SILICO APPROACH

Florian Fischer[1], Veronika Temml[1], Daniela Schuster[1]

[1]Institute of Pharmacy, Department of Pharmaceutical and Medicinal Chemistry, Paracelsus Medical University, Salzburg, Austria; Contact: florian.fischer@pmu.ac.at

Objective

Janus kinases (JAKs) are a group of tyrosine kinases that play a crucial role in transmitting signals from cytokines and growth factors. They are involved in many essential biological processes, including immune function, inflammation, and haematopoiesis. The JAK- signal transducer and activator of transcription protein (STAT) pathway is activated when a signalling molecule, such as cytokines or growth factors, binds to a cell surface receptor. This triggers the receptor's phosphorylation, which then activates JAK proteins. Activated JAK proteins phosphorylate STATs, enabling them to combine and move into the nucleus to regulate gene expression. Dysregulation caused by mutations in the JAK signalling pathway was observed in several autoimmune diseases and cancer (1). Therefore, there is great interest in finding new inhibitors for the four JAK subtypes JAK1, JAK2, JAK3 and TYK2.

Methods

The project aims to create high-quality pharmacophore models in LigandScout version 4.4.5 (2). Pharmacophore modeling is a technique used to identify the key physicochemical interaction features necessary for binding to a protein target, these feature patterns can then be used to screen for molecules with similar interaction patterns. This approach is often used in virtual screening to preselect potential drug candidates for further testing. Structure-based models are derived from well-resolved three-dimensional crystal structures of the different JAK subtypes. Ligand-based pharmacophore models are generated by aligning known active molecules in 3D and determining common elements in their pharmacophores. For each Janus kinase subtype both structure- and ligand-based modeling is used to find new potential inhibitors. High quality modeling requires the generation of datasets of active and inactive compounds for each subtype from the literature. Datasources include ChEMBL, PubChem, Protein Data Bank and the original literature.

Results

Created pharmacophore models for each subtype were optimised to find a maximal number of active compounds and a minimal number of inactive substances and decoys. Quality metrics for each model are calculated to evaluate model performance in accordance with the literature (3).

Conclusions

In conclusion, pharmacophore modelling is successfully used to develop high-quality models for each JAK subtype. Training and theoretical validation of these models using comprehensive datasets enable accurate prediction of active and inactive compounds. These models can be used to find novel lead structures targeting different JAK subtypes and potentially lead to the development of more effective treatments for diseases such as rheumatoid arthritis and psoriasis (1).

References

1. Roskoski R. Janus kinase (JAK) inhibitors in the treatment of neoplastic and inflammatory disorders. Pharmacol Res. 2022 Sep;183:106362.
2. Wolber G, Langer T. LigandScout: 3-D pharmacophores derived from protein-bound ligands and their use as virtual screening filters. J Chem Inf Model. 2005;45(1):160-9
3. Zell L, Lainer C, Kollár J, Temml V, Schuster D. Identification of Novel Dopamine D2 Receptor Ligands-A Combined In Silico/In Vitro Approach. Molecules. 2022 Jul 11;27(14):4435. doi: 10.3390/molecules27144435. PMID: 35889317; PMCID: PMC9318694

Komplementregulation in synovialen Fibroblasten unter dem Einfluss des SARS-CoV-2-Nukleokapsid Proteins

Vincent Franke[1], Sophie Meyer[1], Gundula Schulze-Tanzil[1], Tobias Braun[2], Maria Kokozidou[1], Theodor Fischlein[2], Sandeep Silawal[1]

[1]Institut für Anatomie und Zellbiologie, Paracelsus Medizinische Privatuniversität, Nürnberg, Deutschland; [2]Klinik für Herzchirurgie, Paracelsus Medizinische Privatuniversität, Nürnberg, Deutschland; Contact: vincent.franke@stud.pmu.ac.at

Objective
Case reports are available showing patients developing symptoms of reactive arthritis during and after SARS-CoV-2 infection. In case of joint inflammation, immunological processes are triggered in joint cells including synoviocytes. During an SARS-CoV-2 infection, all viral components, including the nucleocapsid protein (NP), are found abundantly in the system. The aim of this study is to analyze the impact of NP as well as tumor necrosis factor (TNF)-α on human synoviocytes in terms of complement and cytokine regulation.

Methods
Immortalized synoviocytes (K4IM, non-arthritic) and immortalized rheumatoid arthritic synoviocytes (HSE) were used in this study. As stimulating agents served recombinant SARS-CoV-2 NP and TNFα. Cell vitality, proliferation, gene and protein expression were analysed.

Results
The live-dead staining did not show any adverse effect on the stimulated or non-stimulated cells. Almost 2-fold higher proliferation in HSE in comparison to K4IM was shown. In general, HSE displayed lower gene expression of regulatory complement factors such as CD46, CD55, CD59, C5aR1 as well as TNFα but a higher expression of interleukin (IL)-6 gene. NP alone induced a slightly higher C5aR1 gene expression in K4IM in comparison to controls, however TNFα, especially in combination with NP showed drastically higher TNFα gene expression in K4IM. CD35 gene expression could not be detected.

Conclusions
NP impairs HSE cell proliferation, which could be interpreted as a protective effect on RA synoviocytes. However, compromised immune status and immunomodulatory drugs seem more likely as a cause of increased risk of infection and severe course of COVID-19 infection. NP might show its effects under inflammatory conditions. K4IM vs HSE: HSE has approximately twice the proliferation rate, which matches with hyperplasia and pannus formation in RA-joint, and has lower gene expression of complement factors, as well as TNFα, but increased gene expression of IL-6.

References
1. Zeidler, H., Post-Corona-Virus-Disease-19 arthritis. Manifestation under the clinical picture of a reactive arthritis. Z Rheumatol, 2021. 80(6): p. 555-558.
2. Dombret, S., A. Skapenko, and H. Schulze-Koops, Reactive arthritis after SARS-CoV-2 infection. RMD Open, 2022. 8(2).
3. Janeway Jr, C.A., et al., Immunobiology: The Immune System in Health and Disease. 5th edition. 2001, New York: Garland Science.

Role of Galanin receptors 2 and 3 in polymicrobial sepsis

Antonia Gantschnigg[1,2], Sara Huber[2], Lysanne Lüthje[1], Jonas Strauss[1], Stefanie Gaisbauer[2], Victoria Stefan[2], Klaus Emmanuel[1], Barbara Kofler[2]

[1]Department of General, Visceral and Thoracic Surgery, University Hospital of the Paracelsus Medical University, Salzburg, Austria; [2]Research Program for Receptor Biochemistry and Tumor Metabolism; Department of Pediatrics, University Hospital of the Paracelsus Medical University, Salzburg, Austria; Contact: a.gantschnigg@salk.at

Objective

Sepsis is a global healthcare issue associated with a high morbidity and mortality in critical ill patients [1]. Intra-abdominal infections (IAI) are the second most common focus of sepsis after pulmonary infections and are a common perioperative complication associated with a mortality rate of up to 9.2% [2]. The exact pathogenesis of sepsis and immunological mechanisms have not yet been fully elucidated. The regulatory peptide galanin (GAL) and its receptors (GAL_{1-3}-R) are involved the regulation of immunity and inflammatory processes [3]. In this study, we aimed to elucidate the effect of $GAL_{2/3}$-R on the course of abdominal sepsis.

Methods

Colon ascendens stent peritonitis (CASP) surgery was performed in $GAL_{2/3}$-R knockout (KO) and Wild type (WT) mice. Sham surgery was performed on the control group (laparotomy without perforation of the colon ascendens) to determine surgery-induced effects [4]. After 12, 24 and 72 hours respectively, peritoneal fluid, spleen and blood cells were collected. Detailed phenotyping of myeloid and T-cell populations was performed by flow cytometry.

Results

By comparing CASP versus sham-operated mice, the successful induction of abdominal sepsis was verified at all time points. CASP surgery induced increased macrophages, natural killer (NK), dendritic cells and T-cells in both genotypes compared to the sham group.

Twelve hours after CASP surgery $GAL_{2/3}$-R KO mice showed significant lower levels of neutrophils and NK cells in spleen and neutrophils in blood compared to WT mice. Dendritic cells in the spleen as well as B-cells and CD8+ T-cells in the peritoneal fluid were higher in $GAL_{2/3}$-R KO mice. These results indicate an early initiation of adaptive immune response at the site of inflammation. 24 hours after CASP, lower levels of CD8+ T-cells in blood were observed in $GAL_{2/3}$-R KO compared to WT mice.

In the late phase of sepsis, 72 hours postoperative, blood dendritic cells, Ly6C+ macrophages and monocytes were higher in $GAL_{2/3}$-R KO, lower levels of Ly6C- macrophages and CD3+ T-cells in spleen were observed in the KO animals. This could be due to a lower adaptive immune system in the late phase of sepsis in $GAL_{2/3}$-R KO mice, leading to re-initialization of the innate immune system.

Conclusions

In the early phase of sepsis, $GAL_{2/3}$-R KO mice lacked the characteristic induction of neutrophils and NK cells by the innate immune system, but had an increase of cells involved in early initiation of the adaptive immune responses. In contrast, $GAL_{2/3}$-R KO mice show more cells of the innate immune system in the late phase of sepsis. Future studies will compare mRNA expression of various pro- and anti-inflammatory cytokines in liver, lungs, kidneys and lymph nodes in $GAL_{2/3}$-R KO and WT mice. Furthermore, we will determine the concentration of pro- and anti-inflammatory cytokines in the peritoneal fluid of CASP mice.

References

1. Cecconi et al., Lancet. 2018
2. Sartelli et al., World J Emerg Surg. 2017
3. Koller et al., Sci Rep. 2019
4. Traeger et al. J Vis Exp. 2010

STEM CELL APHERESIS: PREDICTION OF THE STEM CELL YIELD USING PREAPHERESIS CD34 CELL COUNT - COMPARISON BETWEEN THE ALLOGENEIC AND AUTOLOGOUS SETTING

Johannes Gärtner[1]

[1] 5. Medizinische Klinik, Klinikum Nürnberg, Paracelsus Medical University, Nuremberg, Germany; Contact: johannes.gaertner@klinikum-nuernberg.de

Objective
We could previously show in the allogeneic setting, that the prediction of the stem cell yield based on the pre-aphesis peripheral CD34+ cell count is accurate and reliable. This information is essential for planning and executing of the PBSC leukapheresis procedure. We investigated whether this method of prediction is also feasible for the autologous stem cell collection and compared these results with the allogeneic setting.

Methods
We performed 462 allogeneic apheresis and 138 autologous stem cell apheresis in the years 2021 and 2022 , using the Spectra Optia System (Terumo BCT). The calculation of the final CD34 count (CD34+ per kg bodyweight recipient) in the product was based on the CD34+ cell count in peripheral blood before apheresis with the following method: Predicted CD34 x10 /kg = (benchmark collection efficacy x processed blood volume x peripheral CD34 count per µl) / (patient's weight x metric conversion factor). We retrospectively investigated the correlation between the initially predicted and the finally collected CD34+/kg in the product for the allogeneic and the autologous setting. Also, the ratio between predicted and collected stem cell yield (prediction coefficient) was analyzed.

Results
We performed 462 allogeneic (433 volunteer donors) and 138 autologous (97 patients) apheresis procedures during the years 2021-2022. In the allogeneic setting, the median pre-apheresis CD34+ count in the donor´s blood was 67/ul, in the autologous setting the pre-apheresis CD34+ count was only 49,5/ul. The rate of second day apheresis was 6,69% for the allogeneic collections and 39,18% for autologous apheresis. Furthermore, in the autologous setting the additional application of plerixafor was necessary in 21,7% of the cases. No plerixafor was used for the allogeneic apheresis procedure (not approved). For the allogeneic aphereses, the median value of the prediction coefficient of was 1.08, translating into only slight underestimation of the finally collected stem cell count. The Spearman´s correlation coefficient (r) between calculated and actual collected CD34/kg was 0.96 ($p < 0.01$). In the autologous setting the median prediction coefficient was 1,21, representing a slightly higher underestimation rate, the Spearman´s correlation coefficient (r) was 0,95 ($p<0,01$).

Conclusions
In the autologous setting, the method of calculating the stem cell yield based on the pre-apheresis CD34+ count in the peripheral blood is also highly predictive for the number of CD34 cells actual collected, as has been shown for allogeneic apheresis. For the autologous setting, despite a higher rate of low mobilizers, different mobilizing procedures (e.g. mobilizing by chemotherapy, steady state mobilizing, use of Plerixafor) and heavily pretreated patients with different underlying diseases, the accuracy of our calculation method is still very high. This makes planning and adjusting of the apheresis procedure very reliable. For the autologous stem cell apheresis, this predictability is at least as important as in the allogeneic setting, because of a higher risk for low mobilizers, accounting for a second day apheresis and an frequently higher stem cell yield, e.g. for multiple myeloma or germ cell tumors (47% of the patients).

References
1. EBMT 2022, Poster 554

Umbilical catheter placement aided by coronary guidewires

Katarzyna Gendera[1], Stanimir Georgiev[1], Peter Ewert[1], Stefan Eckstein[2], Christoph Fusch[3,4], Niels Rochow[3,4,5]

[1]Department of Paediatric Cardiology and Congenital Heart Defects, German Heart Centre Munich, Technische Universität München, Munich, Germany; [2]Eckstein Design, Munich, Germany ; [3]Department of Pediatrics, Paracelsus Medical University, Nuremberg, Germany; [4]Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada; [5]Department of Pediatrics, University Medical Center Rostock, Rostock, Germany ; Contact: gendera.kasia@gmail.com

Objective
Catheterization of the umbilical cord vessels has proven to be an effective and rapid method for gaining central vascular access in neonates. However, it can be technically difficult and be associated with complications in some patients. Using a coronary guidewire during catheterization of umbilical vessels supports the placement of umbilical catheters and significantly reduces a risk for complications.

Methods
Tests were performed in cath lab in German Heart Center in Munich. The use of a coronary guidewire was attempted in 6 successful ex vivo laboratory tests for catheterization of umbilical vessels in stillborn piglets immediately after birth. Abbott BMW Wire Hi-Torque Balance Middleweight Universal™ Guide Wire 0.014", 190 cm and polyurethane umbilical catheter with a rounded tip (Umbili CathTM) were used. The position of the wire and the position of the catheter were controlled by fluoroscopy (C-arm machine, Philips Azurion ClarityIQ). Also, an angiography was performed.

Results
In all 6 animals it was possible to cannulate the umbilical cord vessels quickly (within maximal few minutes) and without any complications. The position of the catheter was proven by fluoroscopy.

Conclusions
Indication for umbilical catheter placement has become restrictive in the recent time. As a result, safety aspects have become even more relevant. Using of a coronary guide wire as a guiding device during catheterization of the umbilical vessels is a fast and safe method. It allows to gain vascular access with less risk of dangerous procedural complications, which could be life-saving in critically ill patients.

Acknowledgements
No

References
1. n/a

Prevalence and characteristics of metabolic hyperferritinemia in a population-based Central-European cohort

Sophie Gensluckner[1,2], Bernhard Wernly[3], Florian Koutny[4], Bernhard Iglseder[5], Eugen Trinka[6], Vanessa Frey[6], Patrick Langthaler[6], Georg Semmler[7], Christian Datz[3], Lars Stechemesser[1,2], Bernhard Paulweber[1,2], Elmar Aigner[1,2]

[1]Departement of internal Medicine 1, Paracelsus Medical University, Salzburg, Austria; [2]Obesity Research Unit, Paracelsus Medical University, Salzburg, Austria; [3]Department of Medicine, General Hospital Oberndorf, Teaching Hospital of the Paracelsus Medical University, Oberndorf, Austria; [4]Department of internal Medicine 2, Gastroenterology and Hepatology and Rheumatology, Karl Landsteiner University of Health Sciences, University Hospital of St. Pölten, lower Austria, Austria; [5]Department of Geriatric Medicine, Christian Doppler Klinik, Paracelsus Medical University, Salzburg, Austria; [6]Department of Neurology, Christian Doppler University Hospital, Paracelsus Medical University and Centre for Cognitive Neuroscience, Affiliated member of the European Reference Network EpiCARE; [7]Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; Vienna Hepatic Hemodynamic Lab, Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria; Contact: s.gensluckner@salk.at

Objective
Hyperferritinemia (HF) is a common laboratory finding and considered as metabolic HF (MHF) if observed in combination with metabolic diseases like type 2 diabetes mellitus, obesity or fatty liver disease. The definition of the term metabolic HF has been heterogenous, until a consensus statement on the classification and grading of MHF has been published recently. The aim of this study was to apply the definition of MHF in order to provide data on the prevalence and characteristics of MHF in a Central-European cohort.

Methods
This analysis included 6424 participants of the Paracelsus 10.000 study, which is a population-based cohort study including approximately 10.000 randomly selected subjects aged 40-77 from the region of Salzburg, Austria. Participants with HF were divided into three categories according to their level of serum ferritin and were further evaluated for associated metabolic co-morbidities defining the proposed criteria for MHF.

Results
HF was present in 13% (n = 857) of the general population with a clear male preponderance (n = 590, 69% of HF). Within the HF group, 84% (n = 719) subjects fulfilled the metabolic criteria and may therefore be defined as MHF, of which 63% (n = 540) were characterized by a major criterion. In the remaining HF cohort, 56% (n = 179 of 317) were classified as MHF after application of the minor criteria. The prevalence of metabolic co-morbidities was higher in HF subjects and increased along the different grades of HF.

Conclusions
HF is a common finding in the general middle-aged population with approximately 1 in 8 subjects affected. The majority of these is classified as MHF making it by far the most common cause of elevated SF concentrations. The new classification provides useful criteria for defining MHF.

References
1. Valenti L, Corradini E, Adams LA, et al. Consensus Statement on the definition and classification of metabolic hyperferritinaemia. Nat Rev Endocrinol. Published online February 17, 2023. doi:10.1038/s41574-023-00807-6

IMPACT OF ANASTOMOTIC LEAK VS PNEUMONIA ON FAILURE TO RESCUE AFTER TRANSTHORACIC ESOPHAGECTOMY FOR CANCER

Luca Giulini[1], Melissa Kemeter[1], Lucas Thumfart[1], Oliver Koch[2], Michael Grechenig[2], Klaus Emmanuel[2], Wolfgang Hitzl[3,4,5], Attila Dubecz[1]

[1]Department of Surgery, Paracelsus Medical University Nuremberg, Nuremberg, Germany; [2]Department of General, Visceral and Thoracic Surgery, Paracelsus Medical University Salzburg, Salzburg, Austria; [3]Biostatistics and Publication of Clinical Trial Studies, Research and Innovation Management (RIM), Paracelsus Medical University Salzburg, Salzburg, Austria; [4]Department of Ophthalmology and Optometry, Paracelsus Medical University Salzburg, 5020 Salzburg, Austria; [5]Research Program Experimental Ophthalmology and Glaucoma Research, Paracelsus Medical University, 5020 Salzburg, Austria; Contact: luca.giulini84@gmail.com

Objective
Data about failure-to-rescue (FTR) after esophagectomy for cancer and its association with patient and procedure-related risk factors are sparse. Aim of the study was to analyze such aspects, particularly focusing on the impact of pneumonia and anastomotic leak on FTR.

Methods
All patients who underwent a transthoracic esophagectomy for cancer between 2007 and 2022 in two high-volume European centers were prospectively identified. Patients were classified and compared according to the type of operation (open, hybrid-laparoscopic, hybrid-robotic, standard minimally invasive or robotic-minimally-invasive). Failure-to rescue was defined as in-hospital death following a complication. Risk factors for in-hospital mortality were analyzed and identified with a univariable model. Mortality after pneumonia and anastomotic leak were calculated and compared across the groups.

Results
In total, 708 patients were included. Median operative time was 268 minutes (IQR 239-305). There were 355 (50.1%) open, 204 (28.8%) hybrid-laparoscopic, 121 (17.1%) hybrid-robotic, 15 (2.1%) standard minimally-invasive and 11 (1.6%) robotic minimally-invasive procedures. Overall morbidity was 60%, in-hospital mortality 4.8% and failure-to-rescue rate 4.5%. Anastomotic leak, pneumonia, postoperative bleeding, sepsis, pulmonary embolism, arrhythmia and need for blood transfusion were the risk factors significantly associated with in-hospital mortality ($p<0.05$). There was no particular type of operation significantly more associated with mortality ($p=0.42$). Pneumonia and leak associated failure-to-rescue rates did not significantly differ among the groups ($p=0.99$).

Conclusions
Transthoracic esophagectomy for cancer still represents a complex operation with high morbidity and mortality, and challenging postoperative management. Despite their complexity, the use of hybrid, minimally-invasive or robotic methods do not seem to negatively affect the FTR rates. Therefore, efforts should be made to increase the implementation of minimally-invasive esophagectomy, and to improve postoperative care.

References
1. Abdelsattar ZM, Habermann E, Borah BJ, Moriarty JP, Rojas RL, Blackmon SH. Understanding Failure to Rescue After Esophagectomy in the United States. Ann Thorac Surg. 2020 Mar;109(3):865-871.

Tendinogenesis via cyclic stretch of 3D printed cocultured tendon/stem cell scaffolds in vitro

Clemens Gögele[1], Jens Konrad[2], Bernd Hoffmann[2], Christian Werner[1], Maria Kokozidou[1], Kerstin Schäfer-Eckart[3], Gundula Schulze-Tanzil[1]

[1]Institute of Anatomy and Cell Biology, Paracelsus Medical University, Nuremberg and Salzburg, 90419 Nuremberg, Germany; [2]Institute of Biological Information Processing: IBI-2, Forschungszentrum Jülich, 52425 Jülich, Germany; [3]Bone marrow Transplantation Unit, Medizinische Klinik 5, Klinikum Nürnberg, Paracelsus Medical University, 90419 Nuremberg, Germany; Contact: clemens.goegele@pmu.ac.at

Objective
The performance of movements can be massively impaired after tendon injuries. The self-healing and regeneration capacities of tendons are greatly reduced. However, in order to prevent serious consequences, tissue engineered constructs could be used in the future. In the planned basic research project, the effect of cyclic stretch on tendon cells as well as on human mesenchymal stem cells (hMSCs) in a 3D printed cocultured tendon scaffold.

Methods
At the beginning, we will try to extract the dura mater of undamaged as possible from the skull halves of pigs in order to subsequently perform the dissections according to an established protocol (1), so that a cell-free scaffold can be produced. The decellularized dura will be colonized statically with primary hMSCs for 24 hours, thus forming the "carpet pad" for the future scaffolds. Subsequently, by means of 3D Bioprinter to print rat Achilles tendon cells onto the revitalized dura tissue and cultivate them statically for another 24 hours. The scaffolds should then be removed from the stretched position and rolled up in a wrap roll-like manner. The resulting coculture scaffolds will be subjected to a dynamic motion cycle in a 3D tissue stretcher for 8 days. Control scaffolds are cultured statically for the same period in parallel. After the "training program" extensive analyses (immunocytochemical and histological staining, quantitative measurement of DNA and sulfated glycosaminoglycan content, detection of tendon-associated matrix components on protein and gene level) will be performed to characterize the tendinogenesis.

Results
The dura mater could be dissected in its entirety from the skull halves without injuring or damaging the structure. The vitality assay shows that due to a gentle preparation of the dura, the cells are still vital and only a few dead cells can be found in the tissue. The vitality was above 50%. Decellularization could be performed according to established protocol. Histological staining (Alcian blue, hematoxylin eosin, resorcinol fuchsin stain and Sirius Red) show that the cells were successfully removed from the tissue and that neither the tissue structure nor extracellular matrix components were destroyed. Quantitative analyses confirmed the reduction in DNA content after decellularization and a maintenance of the sulfated glycosaminoglycan content.

Conclusions
It could be shown that a dura half can be gently removed in its entirety, so that a sufficiently large "scaffold base" is available for the further experiments. Since decelluarization was successfully performed, revitalization using hMSCs and the application of the wavelike printed Achilles tendon cells can be started.

Acknowledgements
The work is financially supported by NÜRNBERGER Krankenversicherung AG.

References
1. Gögele C, Schwarz S, Ondruschka B, Hammer N, Schulze-Tanzil G. Decellularized Iliotibial Band Recolonized with Allogenic Homotopic Fibroblasts or Bone Marrow-Derived Mesenchymal Stromal Cells. Methods Mol Biol. 2018;1577:55-69. doi: 10.1007/7651_2017_30. PMID: 28488244.

Chondrogenesis in 3D printed bioglass/hydrogel scaffolds for cartilage regeneration

Clemens Gögele[1], Nils Fleischmann[1], Silvana Müller[2], Sven Wiltzsch[2], Armin Lenhart[2], Christian Werner[1], Kerstin Schäfer-Eckart[3], Gundula Schulze-Tanzil[1]

[1]Institute of Anatomy and Cell Biology, Paracelsus Medical University, Nuremberg and Salzburg, 90419 Nuremberg, Germany; [2]Faculty of Materials Science, Technische Hochschule Nürnberg Georg Simon Ohm, 90489 Nuremberg, Germany; [3]Bone marrow Transplantation Unit, Medizinische Klinik 5, Klinikum Nürnberg, Paracelsus Medical University, 90419 Nuremberg, Germany; Contact: clemens.goegele@pmu.ac.at

Objective
The self-healing capacities of cartilage tissue are greatly reduced after injury, which means that cartilage defects can expand very rapidly and cause the onset of osteoarthritis (OA) disease if left untreated. For this reason, there is still an urgent need to develop a new, cost-effective, implantable and chondroinductive (cartilage formation inducing matrix) with human mesenchymal stromal cells (hMSCs). Our team developed a completely novel and bioactive glass (CAR12N) for cartilage regeneration (1). In contrast to conventional bioglasses, which support bone regeneration based on their ability to form hydroxyapatite, our glass exhibits a more rapid degradation rate. The previously unresolved issue of continuous ion leakage and the associated stimulatory effects on hMSCs, will be addressed in this project. This basic research project focuses on cartilage development (chondrogenesis) by hMSCs in 3D printed hydrogel scaffolds containing CAR12N bioactive glass spheres.

Methods
Preliminary experiments will be performed with the murine fibroblast cell line L929. The scaffolds are composed of a hydrogel (mixture of alginate, methylcellulose and CAR12N bioactive glass spheres) and hMSCs (3.6 x 10^6 cells/mL hydrogel), fabricated via a "layer-to-layer" process using 3D printer (Bio X, CELLInk) and have a porosity of 70%. After scaffold printing, these are cross-linked in a 100 mM calcium chloride solution for 10 minutes and then statically cultured in different culture media. After long-term cultivation of up to 35 days, extensive analyses (vitality assay, metabolic activity assay, DNA and sulfated glycosaminoglycan contents, chondrogenic matrix components on protein and gene level) will be performed to adequately assess the progress of chondrogenesis.

Results
In the first set of experiments, it was shown that the L929 and hMSCs together with bioactive glass spheres could be printed into 3D scaffolds in a hydrogel of alginate and methylcellulose. The qualitative viability assay shows that the cells can survive from the printing process (0 h) up to 35 days in all areas (around the pores, in the center and at the edge) of the scaffold. No change in scaffold size, premature degradation or accelerated degradation rate of the glass spheres could be detected. The glass spheres were able to be printed and were homogenously distributed inside the scafflolds.

Conclusions
For this project, a completely new protocol for the production of 3D printed scaffolds first had to be established. Initial test approaches with two different cell types have already shown that the printing parameters meet the cellular requirements. Further optimization steps and the actual project implementation are currently the subject of research.

Acknowledgements
The work is financially supported by the Förderverein der Paracelsus Medizinischen Privatuniversität.

References
1. Gögele C, Wiltzsch S, Lenhart A, Civilleri A, Weiger TM, Schäfer-Eckart K, Minnich B, Forchheimer L, Hornfeck M, Schulze-Tanzil G. Highly porous novel chondro-instructive bioactive glass scaffolds tailored for cartilage tissue engineering. Mater Sci Eng C Mater Biol Appl. 2021 Nov;130:112421. doi: 10.1016/j.msec.2021.112421. Epub 2021 Sep 10. PMID: 34702508.

IMPROVING MUSCLE STEM CELL INTEGRATION FOR ENHANCED REGENERATION

Christian Grabner[1,3], Ariane Benedetti[1,3], Pasquale Romanelli[1,2], Marco Thurner[2], Rainer Marksteiner[2], Sebastien Coulliard-Despres[1,3,4]

[1]Institute of Experimental Neuroregeneration, Paracelsus Medical University, Salzburg, Austria; [2]Innovacell AG, Innsbruck, Austria; [3]Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Austria; [4]Austrian Cluster for Tissue Regeneration, Austria; Contact: christian.grabner@pmu.ac.at

Objective

Satellite cells (SC) are a promising tool for the treatment of injured or diseased skeletal muscles. Isolated from a muscle biopsy, SC can be re-introduced into the muscle and engraft into the host tissue. Despite this remarkable capacity, technical limitations hinder the development of regenerative therapies based on SC transplantation. First, the number of SC that can be isolated from muscle biopsies is very small. Moreover, the extraction of SC from their niche leads to their activation and differentiation along the myogenic lineage. The latter cause a progressive loss of regenerative capacity since myoblasts are not capable of self-renewal and cannot replenish the stem cell pool. To improve the efficiency of engraftment of in vitro expanded SC (called skeletal muscle derived cells, or SMDC), we assessed if we could add more cells to the muscle environment and increase regeneration via repeated implantation. Physical exercise is known to stimulate SC activation and muscle regeneration. In our experimental setup, we provided access to a running wheel immediately after transplantation with the aim to facilitate activation and fusion of SMDC with the host muscle fibers. Finally, we investigated whether transplanted SMDC could re-integrate the pool of SC and support future regenerative processes.

Methods

One million transgenic mouse donor SMDC expressing reporter genes (TdTomato und firefly luciferase) were used to track cells in vivo and ex vivo after their injection into the tibialis anterior (TA). Engraftment was monitored by repeated measurement of the bioluminescence reporter for several weeks after transplantation. Immunohistology and RNAscope were performed to analyse reporter SMDC engraftment and expression of specific satellite cell markers. SMDC re-implantations were conducted 10 weeks after to verify whether more SMDC could be integrated over time. Running wheels for voluntary training were also provided to a group of mice immediately after transplantation for a period of 10 weeks to assess the impact on SMDC integration. Finally, cardiotoxin was administered to elicit a muscle injury destroying existing transgenic myofiber and forcing regeneration to verify if previously transplanted SMDC could integrate the stem cell pool and lead to newly formed TdTomato+ myofiber.

Results

Two re-implantations led to a significant higher bioluminescence signal intensity in vivo and a significant increment in the number of TdTomato+ myofibers as compared to the single SMDC transplantation The co-localization of TdTomato and Pax7 mRNA 30 weeks after SMDC transplantation revealed maintenance of myogenic regenerative capability. Physical exercise had no overt effect on SMDC engraftment at first sight. The induction of a muscle injury led to the generation of newly formed transgenic myofibers.

Conclusions

Re-implantations of SMDC had a positive impact on the regenerative capacity, seen as an increment of TdTomato+ myofiber and TdTomato RNA containing nuclei, and increase of total engraftment. Physical exercise in form of voluntary running had no beneficial effect on muscle regeneration at first sight and requires further examinations. Furthermore, our experimental model demonstrated, that transplanted transgenic SMDC can replenish the stem cell pool available for regeneration after future injuries.

References

1. Hekmatnejad, B., Rudnicki, M.A., 2022. Transplantation to study satellite cell heterogeneity in skeletal muscle. Front. Cell Dev. Biol. 10, 902225. https://doi.org/10.3389/fcell.2022.902225

Markers from standard magnetic resonance imaging in the early post-injury phase are predictive of neurological outcome in individuals with traumatic cervical SCI: A prospective longitudinal study.

Lukas Grassner[1,2,3,4,5], Iris Leister[1,2,3,4], Florian Högel[1,2], Orpheus Mach[1,2], Doris Maier[1,2], Andreas Grillhösl[6]

[1]ParaMove, SCI Research Unit, BG Trauma Center Murnau, Murnau, Germany, Paracelsus Medical University, Salzburg, Austria; [2]Spinal Cord Injury Center, Clinical Research Unit, BG Trauma Center Murnau, Murnau, Germany; [3]Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg, Austria; [4]Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University, Salzburg, Austria; [5]Department of Neurosurgery, University Hospital Salzburg, Paracelsus Medical University, Salzburg, Austria; [6]Department of Radiology, Neuroradiology and Interventional Radiology, BG Trauma Center Murnau, Murnau, Germany; Contact: iris.leister@bgu-murnau.de

Objective
In recent decades, advanced magnetic resonance imaging (MRI) techniques, that are able to quantify the extent of injury in the spinal cord, evolved. The field of spinal cord injury (SCI) research is in need for biomarkers that are able to objectively quantify the extent of injury, measure a therapeutic effect, or predict outcome.
In this study, we aimed to investigate 1) if digital biomarkers from standard, as well as advanced MRI in the early post-injury phase are predictive of the neurological outcome, and 2) to study the trajectory of those markers over time from acute to chronic stages of SCI.

Methods
We conducted a prospective, longitudinal cohort study in 52 patients with acute cervical SCI, who were treated at Trauma Center Murnau (Germany) between 2016-2021. All participants underwent at least three out of four study related examinations including an MRI, as well as a neurological assessment. Study visits took place between days 1-5, days 16-40, days 70-98, and days 300-546.

Results
Unbiased recursive partitioning for the endpoint of lower extremity motor function divided the cohort in two groups according to their BASIC score at acute I. Individuals with a BASIC score of 2 and below showed significantly higher lower extremity motor scores (LEMS), compared to patients with BASIC score above 2. Patients with hyperintensities in the dorsal column had lower pin-prick scores, and the presence of intramedullary hemorrhage was associated with lower motor and sensory function. Linear mixed models revealed a significant decrease of fractional anisotropy, and a significant increase of mean diffusivity values in the reference area C0-C4 over time until the chronic stage of SCI.

Conclusions
Simple MRI markers in the early post-injury phase are predictive of neurological outcome in individuals with traumatic cervical SCI.

Acknowledgements
This project was funded by the German Statutory Accident Insurance (Deutschen Gesetzliche Unfallversicherung e.V., DGUV).

References
1. Rutges JPHJ, Kwon BK, Heran M, Ailon T, Street JT, Dvorak MF. A prospective serial MRI study following acute traumatic cervical spinal cord injury. Eur. 2017;26:2324–32.
2. Haefeli J, Mabray MC, Whetstone WD, Dhall SS, Pan JZ, Upadhyayula P, et al. Multivariate analysis of MRI biomarkers for predicting neurologic impairment in cervical spinal cord injury. Am J Neuroradiol [Internet]. 2017 Mar 1 [cited 2021 Mar 17];38(3):648–55. Available from: /pmc/articles/PMC5671488/
3. Shanmuganathan K, Zhuo J, Chen HH, Aarabi B, Adams J, Miller C, et al. Diffusion Tensor Imaging Parameter Obtained during Acute Blunt Cervical Spinal Cord Injury in Predicting Long-Term Outcome. J Neurotrauma [Internet]. 2017 Nov 1 [cited 2019 Oct 7];34(21):2964–71.

Outpatient psychopharmacological treatment of suicidal adolescents with severe MDD

Isabel Hach[1], Thomas Bertsch[2], Patrick Nonell[3]

[1]Education and Science, Paracelsus Medical University, Nuremberg, Germany; [2]Institute of Clinical Chemistry, Laboratory and Transfusion Medicine Paracelsus Medical University, Nuremberg, Germany; [3]Child and Adolescent Psychiatry and Psychotherapy, Paracelsus Medical University, Nuremberg, Germany; Contact: isabel.hach@klinikum-nuernberg.de

Objective
Depressive disorders often start in childhood and adolescence. Major depressive disorders (MDD) in adolescents are highly recurrent. MDDs are episodic, about 10% become chronic. Adolescents suffering from depressive disorders show an increased risk of suicidal thoughts and suicide. Antidepressant drug therapy with serotonine reuptake inhibitors (SSRIs) is a recommended treatment option for severe and persistent depressive disorders. In Germany, there is only the SSRI fluoxetine approved for treatment of MDD in adolescents. We investigated the gender-specific prevalence of MDD in suicidal adolescents, the outpatient psychotropic treatment of adolescents with severe and/or persistent depressive disorders, and discuss possibilities of therapy improvement.

Methods
The urine presence and plasma levels of prescribed psychotropic drugs of adolescents, hospitalized due to their acute suicidality, but without a known actual suicide attempt (i.e. no acute intoxication or serious self-injuries), were investigated routinely between 01.03.2017 and 31.01.2018. Urine and blood samples were taken at the beginning of closed inpatient admission, i.e. the results of the laboratory analysis reflect outpatient drug intake. The serum levels of psychopharmacological medication (particularly antidepressants, second generation antipsychotics SGAPs) were measured (therapeutic drug monitoring, TDM). This retrospective study was approved by the local institutional review board (IRB 2022_022). Statistical analysis was carried out anonymously using methods of descriptive analysis and procedures on sample comparisons.

Results
Our sample consists of 231 cases (males: N=54; females: N=177, ratio: 1:3,3), aged 12-17 years (average age: 15,4 years). The most prevalent psychiatric diagnoses were (recurrent) moderate or severe depressive episodes (57%) and adjustment disorders (24%) Females were significantly more often diagnosed with depression than males ($p < .0001$). We found 28 cases (males: N=3; females: N=25) fulfilling criteria of severe MDD (i.e. ICD 10 diagnoses: F32.3, F33.1, F33.2). Severity of MDD was not reflected in more antidepressant (on-label) medication. Seven female cases used fluoxetine at admission (fluoxetine levels: 3 supratherapeutic, 3 therapeutic). Seven cases received only antipsychotic agents as monotherapy. Seven cases showed no psychotropic medication.

Conclusions
Depression in our sample is female, severe depression shows a high prevalence in adolescents. The most often found adverse drug reaction (ADR) in adolescents with psychotropic medication is suicidality (1). Our results suggest that there are several opportunities for therapy-improvement of severe depressed adolescents, e.g.: TDM at the beginning and as well in the course of a hospital stay could be used to start a causal and recommended (on-label) therapy (drug treatment and/or psychotherapy). TDM could be used to discuss with affected patients (and their parents) all therapeutic options (e.g. deprescribing, if actual therapy is only therapy of symptoms) and help them to put the best treatment in practice.

Acknowledgements
This study was part of the work „suicidality and suicidal methods in adolescence“ (PI: H. Braun-Scharm) that was supported by the Manfred Roth Stiftung.

References
1. Hetrick SE et al. New generation antidepressants for depression in children and adolescents: a network meta-analysis. Cochrane Database Syst Rev. 2021 May 24;5(5):CD013674. doi: 10.1002/14651858.CD013674.pub2. PMID: 34029378; PMCID: PMC8143444.

Body composition in newborns: A comparison between bioelectrical impedance analysis and air displacement plethysmography

Victoria Hagen[1], Niels Rochow[1], Lennart Lücke, Katja Knab[1], Stefan Schäfer[1], Jasper Zimmermann[1], Stephanie Lohmüller-Weiß[1], Anastasia Meis[1], Kerstin Simon[1], Christoph Fusch[1]

[1]Department of Pediatrics, Paracelsus Medical University, Nuremberg; Contact: victoria.hagen@stud.pmu.ac.at

Objective
Optimal and age-appropriate body composition (BC) significantly improves neurological outcomes in preterm infants while preventing risk for metabolic diseases and obesity in later life. Air displacement plethysmography (ADP) is an established (gold)-standard method, for body composition analysis in preterm infants. However, its use is limited to clinically stable infants without respiratory support. Recently released and updated Bioimpedance Analysis (BIA) device allows body composition assessments in early postnatal period. This device has yet to be validated. The aim of this study is to assess the agreement between the methods BIA and ADP, using ADP as a reference method.

Methods
Retrospective analysis of routine body composition assessments using the ADP (PeaPod®, Cosmed, USA) and BIA (BioScan touch i8 - nano, Maltron, UK) in clinically stable infants without respiratory support. PeaPod and BIA body composition assessment for fat mass (FM), percent fat mass (FM%) and fat free mass (FFM) performed at the same day were compared. Statistical analysis was performed using Microsoft Excel 365 (Microsoft Corporation 365) and R Stdio.
Furthermore, it was analyzed whether the trajectory of FM% and FFM measured with the BIA device follows clinical knowledge and literature references.

Results
Body composition data was collected from August 2022 until April 2023 in 204 preterm infants at the University Children's Hospital Nuremberg. 52 infants were measured with the BIA and ADP simultaneous.
N=120 of those simultaneous BIA and ADP measurements show differences in Bland Altman Plot (FM: 64 ± 104 g, FFM: 60 ± 117 g and FM%: 3 ± 4 %). We identified a smaller 95% confidence interval of BIA for FM% (6-7%) compared to ADP (10-12%). Individual measurements of BIA and ADP results for FFM and FM% follow different trajectories. FM% measured with the BIA device showed relatively high values right after birth while leveling off to a constant, relatively low value in the course. This does not match with clinical knowledge and the weight gain these infants showed.

Conclusions
BIA method could potentially be used as complement method when PeaPod is not applicable. Our data however shows that body composition analysis by BIA shows a large discrepancy when correlated with data from ADP. The observed offset indicates that further validation would be needed for BIA before it could be used for clinical decision making.

References
1. Collins CT, Reid J, Makrides M, Lingwood BE, McPhee AJ, Morris SA, Gibson RA, Ward LC. Prediction of body water compartments in preterm infants by bioelectrical impedance spectroscopy. Eur J Clin Nutr. 2013 Jan;67 Suppl 1:S47-53. doi: 10.1038/ejcn.2012.164. PMID: 23299871.

ATR FTIR as tool for quality control of extracellular vesicles

Sophie Haller[1], Alexander Popoff[1], Cristian Tudor Matea[2], Daniela Schuster[1]

[1]Department of Pharmaceutical and Medicinal Chemistry, Institute of Pharmacy , Paracelsus Medical University, Strubergasse 21, 5020 Salzburg, Austria.; [2]Department of Biosciences and Medical Biology , Paris Lodron University Salzburg, Salzburg, Austria.; Contact: sophie.haller@stud.pmu.ac.at

Objective

Extracellular vesicles represent an exciting new research field with great therapeutic and diagnostic potential. Especially when utilyzed for therapeutic purpose a full characterization prior to the definition of quality control parameters is vital. Independent from the origin of EVs, it has become clear that the protein/lipid ration is strongly connected to the purity of the EVs, decreasing with an increase of purity, since most impurites are protein aggregates. Therefore, a method to easily quantify the protein and lipid content is necessary for the quality control. Colorimetric assays like the BCA assay or the sulpho-phospho-vanillin (SPV) assay one option to measure the lipid and protein content. But in contrast the readout of an ATR-FTIR measurement can give information about both protein and lipid content. In order to evaluate the ATR-FTIR for their suitability as a tool for quality control we used monovariate and univariate analysis and colorimetrically determined values as reference.

Methods

Colorimetric assays were used to determine the protein and lipid content of extracellular vesicles (EVs). In parallel an SDS PAGE was conducted with EVs of different purity grades to investigate which proteins are impurities. In addition, ATR-FTIR was used to aquire spectra of the EVs to determine the protein and lipid content by integration of the respective band in the spectra and the obtained values were compored with the colorimetric results. Furthermore, multivariate analysis of the IR-spectra was also used quantify the lipid and protein content and the obtained values were compared to the values from other methods.

Results

With increased purity the protein/lipid ratio is decreasing which also is confirmed by SDS PAGE of the EVs. Therefore, the protein/ lipid ratio can be considered as criteria for quality control. Analysis of the IR spectra confirmed that this technique can be used to quantify the protein and lipid content of EVs. When compared to each other, multivariate analysis using a partial least square regression model gives more accurate values compared to univariate analysis, which is based on simples integration of the respective IR-bands.

Conclusions

Protein and lipid content are valuable parameters to determine purity of extracellular vesicles. FTIR measurement can be used to determine the protein and lipid content of extracellular vesicles, but monovariate analysis using peak areas of the lipid bands or amide I bands can cause inaccuracies. In contrast, multivariate analysis can increase accuracy, but requires a large amount of samples for the calibration.

References

1. Visnovitz T, Osteikoetxea X, Sódar BW, Mihály J, Lőrincz P, Vukman KV, Tóth EÁ, Koncz A, Székács I, Horváth R, Varga Z, Buzás EI. An improved 96 well plate format lipid quantification assay for standardisation of experiments with extracellular vesicles . J Extracell Vesicles . 2019 Jan 29;8(1):1565263. doi : 10.1080/20013078.2019.1565263.
2. Mihály J, Deák R, Szigyártó IC, Bóta A, Beke Somfai T, Varga Z. Characterization of extracellular vesicles by IR spectroscopy : Fast and simple classification based on amide and CH stretching vibrations . Biochim Biophys Acta Biomembr . 2017 Mar;1859(3):459 466. doi : 10.1016/j.bbamem.2016.12.005.
3. Harald Martens,Reliable and relevant modelling of real world data : a personal account of the development of PLS Regression,Chemometrics and Intelligent Laboratory Systems, Volume 58, Issue 2,2001,Pages 85 95,ISSN 0169 7439,https://doi.org/10.1016/S0169 7439(01)00153 8.

WHAT IS A DIAGNOSIS WORTH? MitoCope: psychosocial experience of parents of children with a mitochondrial disease

Emma Hammerl[1], Anna Spitzer[1], Doris Mayr[1], Johannes A. Mayr[1], Elke Tiefenthaler[1], Katja Steinbrücker[1], Beate Priewasser[2], Saskia B. Wortmann[1]

[1]Centre of expertise for mitochondrial diseases (MetabERN), University Children's Hospital Salzburg, Salzburg, Austria; [2]Institute for Early Life Care, Paracelsus Medical University, Salzburg, Austria; University Children's Hospital, Paracelsus Medical University, Salzburg, Austria; Contact: e.hammerl@salk.at

Objective
Reaching a genetic diagnosis of a mitochondrial disease in a child is often perceived as "diagnostic odyssey". Additionally, after receiving a diagnosis, the parents face ongoing uncertainty (1): mitochondrial diseases show a little predictable and relentlessly progressive, individually heterogeneous course with no curative therapies available. Despite the awareness of this burden little is known if and how getting a genetic diagnosis impacts parental psychosocial experience and coping. Additionally nothing is known about the differences between fathers and mothers in this regard. This information however is crucial to improve the holistic management for families affected by mitochondrial diseases.

Methods
After ethical approval, we conducted a semi-structured interview study. Inclusion criteria: genetically proven mitochondrial disease, both parents available for separate interviews, parents fluent in German. The same interviewer conducted all interviews at a location of the parents' choice. These covered the following topics: demographic data (age, profession, income, distribution of care work) experiences and feelings regarding the diagnosis, changes experienced after getting the diagnosis concerning the topics: everyday life, access to financial support and therapies, connecting with other affected families, interaction with family and friends, attitude to prenatal diagnosis and further desire to have children. All interviews were recorded and transcribed. The data were analyzed manually and descriptive.

Results
13 families have been interviewed. The majority of parents felt relieved when finally knowing the cause of their child's symptoms but also felt emotionally burdened, since their hope for cure had been destroyed. The lack of treatment guidelines and the unpredictable future of their child continued fueling this uncertainty. Parents reacted and coped differently. Mothers mostly focused on their role as mother and did the majority of the care work. Whereas fathers sought to understand the disease pathomechanism and expressed concerns about the parental relationship.

Conclusions
Reaching a genetic diagnosis makes a difference for the affected families in many areas. Due to the mitochondrial diseases' nature, the parents remain burdened after the longed-for diagnosis. These data enhance our understanding of the families' needs and enable to improve the holistic management of families with (suspected) mitochondrial diseases during their journey.

References
1. Senger BA, Ward LD, Barbosa-Leiker C, Bindler RC. The parent experience of caring for a child with mitochondrial disease. Journal of Pediatric Nursing. 2016;31(1):32–41. Available from: https://pubmed.ncbi.nlm.nih.gov/26453412/ . Free full text article. PMID: 26453412

THE ENHANCER LANDSCAPE PREDETERMINES THE SKELETAL REGENERATION CAPACITY OF STROMAL CELLS

Sarah Hochmann[1,§], Kristy Ou[2,§], Rodolphe Poupardin[1,§], Michaela Mittermeir[1], Martin Textor[3,4], Salaheddine Ali[3,5,6], Martin Wolf[1], Agnes Ellinghaus[3,4], Dorit Jacobi[3,4], Juri A. J. Elmiger[3,4], Samantha Donsante[7], Mara Riminucci[7], Richard Schäfer[8,9], Uwe Kornak[3,5,6,10], Oliver Klein[3], Katharina Schallmoser[11], Katharina Schmidt-Bleek[3,4], Georg N. Duda[3,4,12], Julia K. Polansky[2,13], Sven Geissler[3,4,14,#,*], Dirk Strunk[1,#,*]

[1]Cell Therapy Institute, Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University (PMU), 5020 Salzburg, Austria; [2]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, BIH Center for Regenerative Therapies (BCRT), T Cell Epigenetics, Augustenburger Platz 1, 13353 Berlin, Germany; [3]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, BIH Center for Regenerative Therapies (BCRT), Augustenburger Platz 1, 13353 Berlin, Germany; [4]Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Julius Wolff Institute (JWI), Augustenburger Platz 1, 13353 Berlin, Germany; [5]Institute for Medical Genetics and Human Genetics, Charité – Universitätsmedizin Berlin, 13353 Berlin, Germany; [6]Max Planck Institute for Molecular Genetics, 14195 Berlin, Germany; [7]Department of Molecular Medicine, Sapienza University of Rome, 00189 Rome, Italy; [8]Institute for Transfusion Medicine and Immunohematology, Goethe University Hospital, German Red Cross Blood Service Baden-Württemberg—Hessen gGmbH, 60323 Frankfurt am Main, Germany; [9]Institute for Transfusion Medicine and Gene Therapy, Medical Center – University of Freiburg, 79106 Freiburg, Germany; [10]Institute of Human Genetics, University Medical Center Göttingen, 37073 Göttingen, Germany; [11]Institute for Transfusion Medicine, PMU, 5020 Salzburg, Austria; [12]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA; [13]German Rheumatism Research Centre (DRFZ), 10117 Berlin, Germany; [14]Berlin Center for Advanced Therapies (BECAT), Charité – Universitätsmedizin Berlin, 13353 Berlin, Germany; Contact: sarah.hochmann@pmu.ac.at

Objective
Multipotent stromal cells are considered attractive sources for cell therapy and tissue engineering. Despite numerous experimental and clinical studies, broad application of stromal cell therapeutics is not yet emerging. A major challenge is the functional diversity of available cell sources

Methods
Here, we investigated the regenerative potential of clinically relevant human stromal cells from bone marrow (BMSCs), white adipose tissue, and umbilical cord compared with mature chondrocytes and skin fibroblasts in vitro and in vivo.

Results
Although all stromal cell types could express transcription factors related to endochondral ossification, only BMSCs formed cartilage discs in vitro that fully regenerated critical-size femoral defects after transplantation into mice. We identified cell type–specific epigenetic landscapes as the underlying molecular mechanism controlling transcriptional stromal differentiation networks. Binding sites of commonly expressed transcription factors in the enhancer and promoter regions of ossification-related genes, including Runt and bZIP families, were accessible only in BMSCs but not in extraskeletal stromal cells.

Conclusions
This suggests an epigenetically predetermined differentiation potential depending on cell origin that allows common transcription factors to trigger distinct organ-specific transcriptional programs, facilitating forward selection of regeneration-competent cell sources. Last, we demonstrate that viable human BMSCs initiated defect healing through the secretion of osteopontin and contributed to transient mineralized bone hard callus formation after transplantation into immunodeficient mice, which was eventually replaced by murine recipient bone during final tissue remodeling.

References
1. Hochmann et al., Science Translational Medicine. 2023 Mar 22;15(688):eabm7477. doi: 10.1126/scitranslmed.abm7477. PMID: 36947595

Sculpting adulthood in the brain networks: A novel map of dormant precursor maturation in cortical and subcortical areas.

Marie-Theres Hochwartner[1,2,3], Bruno Benedetti[1,2,3], Maximilian Reisinger[1,2,3], Ariane Benedetti[1,2,3], Dominika Jakubecova[1,2,3], Gabriele Gabriele[1,2,3], Thomas Weiger[4], Sebastien Couillard-Despres[1,2,3]

[1]Institute of Experimental Neuroregeneration, Paracelsus Medical University, Salzburg, Austria; [2]Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Salzburg, Austria; [3]Austrian Cluster of Tissue Regeneration, Vienna, Austria; [4]Department of Biosciences, University of Salzburg, Salzburg, Austria; Contact: bruno.benedetti@pmu.ac.at

Objective
Dormant neuronal precursors are peculiar cells of the adult mammalian brain, including humans. Their latent post-mitotic immaturity from birth to adulthood precedes a progressive awakening, following largely unresolved mechanisms. Our past work explored the dormant precursor awakening in the murine paleocortex identifying the transition from the precursor to the neuron state during the early adulthood [1–4]. Strikingly, several records hint towards the existence of this precursor cell type in many brain areas. Therefore, we aimed to generate a map to identify the location, density and phenotypes of awakened and matured dormant precursors in the adult and aged brain.

Methods
Our work was carried out in transgenic mice (DCX-CreERT2/fl-EGFP), in which immature dormant precursors were permanently labelled in vivo at different ages and analyzed after maturation, up to age 15 months. The tissue was labelled immunohistologically *ex vivo* and analyzed by confocal microscopy.

Results
In the adult brain, we pinpointed numerous hotspots of adult-neuron maturation. These involved hypothalamic areas, supraoptic nucleus, amygdala, bed nucleus of the stria terminalis, nucleus accumbens and associative cortices. Strikingly, adult matured neurons belonged to heterogeneous phenotypes, each expressing different patterns of proteins, including Tbr1 or oxytocin, vasopressin, tyrosine hydroxylase, GABA or parvalbumin.

Conclusions
Our findings suggest that complex networks of adult matured neurons refine or reshape existing adult brain networks. Considering the age and the regions in which the process occurs, we speculate that the dormant precursors might be crucial for brain maturation and plasticity, shaping cognitive aspects relevant for adult life.

Acknowledgements
This project was possible thanks to the generous support of the Paracelsus Medical University of Salzburg (PMU), in the frame of the PMU Research and Innovation Fund (PMU-RIF) Seed Money for Novel Innovative Ideas and Preparatory Projects (SEED); project number: 2021-SEED-003-Benedetti.

References
1. Rotheneichner P, Belles M, Benedetti B, König R, Dannehl D, Kreutzer C, et al. Cellular plasticity in the adult murine piriform cortex: Continuous maturation of dormant precursors into excitatory neurons. Cereb Cortex. 2018;28(7):2610–21. PMID 29688272.
2. Benedetti B, Dannehl D, König R, Coviello S, Kreutzer C, Zaunmair P, et al. Functional Integration of Neuronal Precursors in the Adult Murine Piriform Cortex. Cereb Cortex. 2019;1–17. PMID 31647533.
3. Coviello S, Benedetti B, Jakubecova D, Belles M, Klimczak P, Gramuntell Y, et al. PSA Depletion Induces the Differentiation of Immature Neurons in the Piriform Cortex of Adult Mice. Int J Mol Sci. 2021;(22):5733. PMID 34072166.
4. Benedetti B, Couillard-Despres S. Why Would the Brain Need Dormant Neuronal Precursors? Front Neurosci. 2022;16(April):1–7. PMID 35464307.

Median length and head circumference gain of neonates : a systematic review

Matthias Hoffmann[1], Efrah Yousuf[2], Hon Yiu So[3], Manfred Voigt[4], Christoph Fusch[5], Niels Rochow[5]

[1]Paracelsus Medical University, Nuremberg; [2]Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada; [3]Deutsches Wachstumszentrum, Berlin; [4]Department of mathematics and statistics, Oakland Univerisity; [5]Klinik für Neugeborene, Kinder und Jugendliche, Universitätsklinik der Paracelsus Medizinischen Privatuniversität; Contact: matthias.hoffmann@stud.pmu.ac.at

Objective

Growth of head circumference and length of preterm infants during the stay at the neonatal intensive care unit (NICU) depends on the nutritional intake and is related to long term outcome. Particularly important for the clinical assessment of preterm infants is a close relationship between head circumference, brain size and neuronal development.
This study is to conduct a systematic review of intrauterine growth charts based on head circumference and birth length at birth.

Methods

This systematic review reports perinatal surveys that report percentile values for length, or head circumference. The search was conducted on publications released between January 1995 and December 2022, utilizing the databases PubMed, Medline, and Web of Sciences.
The search was limited to surveys reporting on more than 10,000 singleton newborns, providing percentile data for both sexes, and covering a gestational age range of 24 to 41 weeks. Additionally, the publications had to present data for at least three major percentiles (either 3rd , 50th, and 97th percentile or 10th, 50th, and 90th percentile) and be written in English or German.
To analyze the median and interquartile range for growth resulting from the specific percentiles the “gamlss package” was used.

Results

The total sample size comprised of data of 10.607.909 neonates from twelve countries
The average length gain was 1.175 cm/week for boys and 1.114 cm/week for girls.
Looking at the graphs we can see that the length-velocity for the lower percentiles (3rd , 10th, 50th) start at a lower growth-velocity and rises up, while the higher percentiles (90th & 97th) start at a higher velocity and decrease with advancing gestational age.
The graphs cross between the 30th and 31st gestational week. Thereafter the lower percentiles possess a higher growth-velocity.
The average head circumference was 0.761 cm/week for boys and 0.754 cm/week for girls . The percentiles of the head circumference growth is stabile until week 31.

Conclusions

Growth of average length and head circumference derived from the 50th percentiles of a large sample size can be used as reference targets for growth of postnatal length and head circumference. Errors can even lead to misclassifications of newborns to the wrong percentile. We cannot specify, which effects determine our data. Although we included data from nine different countries in our study, the results may not be representative for all populations.
Lower percentiles of length und head circumference show similar growth percentiles. Head circumference was less effected which could be explained from postnatal growth restrictions by which first weight, then length and head circumference.

References

1. https://pubmed.ncbi.nlm.nih.gov/32662526/

PUREBONE - A REALISTIC BONE SURROGATE FOR BIOMECHANICAL IMPLANT TESTING

Marianne Hollensteiner[1], Sabrina Sandriesser[1], Peter Augat[1]

[1]Institute for Biomechanics, BG Unfallklinik Murnau, Murnau, Germany and Paracelsus Medical University, Salzburg, Austria; Contact: marianne.hollensteiner@bgu-murnau.de

Objective
As surgical treatment of osteoporotic femur fractures remains challenging, the development of new implants and their biomechanical testing is crucial. In addition to human specimens, epoxy resin-based artificial bones are considered the gold standard for mechanical testing of osteosyntheses although these do not realistically replicate the interface between bone and implant and provide constructs with excessive stability. Previously, we have developed and validated synthetic bone materials based on polyurethane. Synthetic open-cell cancellous bone exhibits a realistic morphological structure as well as compression behavior like human femoral cancellous bone [1]. A custom-made cortical material has been validated against human cortical bone in screw cut-out experiments [2]. The aim of this study was, to combine the previously validated cancellous and cortical materials into osteoporotic femur models (PuReBone) and to validate them mechanically in comparison to human specimens.

Methods
In a material testing machine, eight custom made PuReBone and two epoxy-based synthetic femora (#3406, Pacific Research Lab., Vashon, US) were tested under four-point bending (AP: anterior-posterior, ML: medial-lateral) and under axial loading. To achieve comparability to the results with human osteoporotic subjects (n=5, [3]) , the mechanical tests were adopted from study by Gluek et al. [3]. To investigate significant differences between groups, analysis of variance of pooled data followed by Tukey post hoc test was performed.

Results
The bending stiffness of human osteoporotic bone (AP: 71 ± 9 Nm^2, ML: 69 ± 43 Nm^2) was slightly lower than that of PureBone (AP: 121 ± 8 Nm^2, ML: 123 ± 14 Nm^2) but showed no statistical differences (AP: $p = 0.91$, ML: $p = 0.41$). Commercially available epoxy-based bones showed significantly higher mean AP (326 Nm^2) and ML (251 Nm^2) bending stiffnesses. In case of axial stiffness, no statistical differences were found between the custom made PureBone (587 ± 101 N/mm) and human osteoporotic bone (419 ± 169 N/mm, (6)) ($p=0.85$). The mean axial stiffness of the epoxy-based bones was more than five times higher than human osteoporotic bones (2768 N/mm).

Conclusions
In addition to the previously validated morphology and mechanics of the synthetic cancellous and cortical bone components, the novel polyurethane-based PuReBone femurs showed satisfactory similar behavior to human osteoporotic bones in bending and axial loading. By adjusting the amount of fillers and by using patient-specific casting molds, the variability of the human population can be better taken into account in biomechanical testing in the future. Furthermore, to provide a reasonable alternative to synthetic epoxy bones, the newly developed PureBone realistically replicates the properties of human osteoporotic bone and is more suitable for biomechanical implant testing.

Acknowledgements
MH and PA acknowledge the funding by the Austrian Science Fund (FWF): T1141-B and by PMU research fund – PMU FFF- A-20/01/038-HOA.

References
1. Klinger et al., J Mech Behav Biomed Mater, 129:105146, 2022.
2. 2. Hollensteiner et al., J Mech Behav Biomed Mater, 117:104405, 2021.
3. 3. Gluek et al., J Biomech, 111:110018, 2020.

A system for the identification and characterization of lead compounds against chronic itch in EB

Dorothee Hölzl[1], Roland Wagner[1], Michael Wiessner[1], Andreas Friedrich[1,2], Anita Jäger[3], Jörg von Hagen[4], Silvia Dossena[5], Johann Bauer[1]

[1]Department of Dermatology and Allergology, University Hospital of the Paracelsus Medical University, 5020 Salzburg, Austria; [2]Department of Biosciences, University of Salzburg, 5020 Salzburg, Austria; [3]Merck KGaA, Frankfurter Str. 250, 64271 Darmstadt, Germany; [4]ryon—GreenTech Accelerator Gernsheim GmbH, Mainzer Str. 41, 64579 Gernsheim, Germany; [5]Institute of Pharmacology and Toxicology, Paracelsus Medical University, Salzburg, Austria; Contact: do.hoelzl@salk.at

Objective

Epidermolysis bullosa (EB) designates a highly diverse group of inherited skin disorders, resulting from mutations in genes encoding structural proteins of the skin. Itch is one of the most prominent symptoms across all EB subtypes. Serotonin (5-HT) has been identified as a potent inducer of serotonergic itch, a histamine-independent form of chronic itch. Concomitantly, HTR7 (5-HT7 receptor) and TRPA1 (transient receptor potential ankyrin 1) channel were established as key mediators of chronic itch in mouse models of atopic dermatitis. Given its emerging role in the development of chronic itch, targeting HTR7 signaling, which leads to elevation of intracellular cAMP and Ca^{2+} levels, may hold promise for alleviating itch in EB. The scope of this work is to establish an experimental setup – including patch clamp whole-cell recordings and the determination of intracellular cAMP and Ca^{2+} levels – to test different substances for their potential to abrogate itch-related signaling.

Methods

HEK293 Phoenix cells were transiently transfected with HTR7 and TRPA1 expression constructs using the calcium-phosphate-method. Nystatin perforated patch clamp whole-cell recordings and measurement of intracellular cAMP and Ca^{2+} levels were conducted 48 hours post-transfection.

Results

Patch clamp whole cell recordings revealed that TRPA1 Ca^{2+} currents can be activated and blocked by channel-specific compounds Allylisothiocyanat (AITC) and A-967079. Furthermore, HTR7 agonist LP44 produced measurable currents that were abrogated upon addition of TRPA1 blocker. Fluorescence-based measurement of intracellular Ca^{2+} levels validated these results. Moreover, quantification of intracellular cAMP levels showed that HTR7 is constitutively active when ectopically expressed in HEK293 cells. Despite the constitutive activity of HTR7, we were able to validate different compounds as agonists, antagonists, or inverse agonists, including methiothepin, an inverse HTR7 agonist.

Conclusions

Targeting the HTR7-TRPA1 pathway constitutes a promising opportunity to alleviate itch in EB. An experimental setup was established to test different compounds for their potential to abrogate itch-related signaling. Since TRP channels are key mediators in the transmission of itch and are downstream of multiple receptors, our setup can easily be expanded to study additional pathways associated with itch.

References

1. Papanikolaou M, Onoufriadis A, Mellerio JE, Nattkemper LA, Yosipovitch G, Steinhoff M, et al. Prevalence, pathophysiology and management of itch in epidermolysis bullosa*. 2021;184(5):816-25.
2. Morita T, McClain SP, Batia LM, Pellegrino M, Wilson SR, Kienzler MA, et al. HTR7 Mediates Serotonergic Acute and Chronic Itch. Neuron. 2015;87(1):124-38.
3. Mack MR, Kim BS. The Itch-Scratch Cycle: A Neuroimmune Perspective. Trends in immunology. 2018;39(12):980-91.
4. Romero G, Pujol M, Pauwels PJ. Reanalysis of constitutively active rat and human 5-HT7(a) receptors in HEK-293F cells demonstrates lack of silent properties for reported neutral antagonists. Naunyn-Schmiedeberg's archives of pharmacology. 2006;374(1):31-9.

Tet-controlled gene expression in Stenotrophomonas maltophilia

Rebecca Horch[1,2], Diana Rasp[3], Ronald Ebbert[2], Joerg Steinmann[1], Ulrich E. Schaible[4], Uwe Mamat[4], Ralph Bertram[1]

[1]Institute of Clinical Hygiene, Medical Microbiology and Infectiology, Klinikum Nürnberg, Paracelsus Medical University, Nuremberg, Germany; [2]Technische Hochschule Nürnberg Georg Simon Ohm, Faculty of Applied Chemistry, Nuremberg, Germany; [3]Study Program in Human Medicine, Paracelsus Medical University, Nuremberg, Germany; [4]Department of Cellular Microbiology, Program Area Infections, Research Center Borstel, Leibniz Lung Center, Leibniz Research Alliance INFECTIONS, Borstel, Germany; Contact: ralph.bertram@klinikum-nuernberg.de

Objective

Stenotrophomonas maltophilia is increasingly recognized as an underscored nosocomial pathogen among the Gram-negative bacteria (1). Intrinsic resistance to different classes of antibiotics makes treatment of infections challenging. A deeper understanding of *S. maltophilia* physiology and virulence requires molecular genetic tools. We here describe the implementation of tetracycline-dependent gene regulation (*tet*-regulation (2)) in this bacterium.

Methods

A *tet*-regulatory region from transposon Tn*10* was cloned into a broad host-range plasmid and was tested in *S. maltophilia* with a *gfp* variant as a quantifiable reporter. Also, the *rmlBACD* operon, encoding factors for the synthesis of O-antigen, was cloned into a plasmid to obtain *tet*-control in an *S. maltophilia rml* deletion mutant. Plasmids were introduced into strain K279a *b*y triparental mating using one *E. coli* donor strain and one *E. coli* helper strain. Synthesis of O-antigen was tested by SDS-PAGE of purified lipopolysaccharide (LPS) and silver-staining or Western-Blot.

Results

In case of the GFP reporter, fluorescence intensity was directly correlated to the concentration of the inducer anhydrotetracycline (ATc) and the duration of induction. When regulation of the *rml* operon was investigated, the LPS pattern was similar to that of wild-type *S. maltophilia* in presence of ATc, whereas without inducer, less and apparently shorter O-antigen chains were detected.

Conclusions

Our findings underscore the functionality and usefulness of the *tet*-system for gene regulation, and prospectively, the validation of targets for new anti-*S. maltophilia* drugs.

Acknowledgements

We are grateful to the Manfred-Roth-Stiftung for financial support of this project. We further acknowledge support from the Leibniz Association (grant SAS-2021-1-FZB of the Leibniz Research Alliance INFECTIONS in an Urbanizing World - Humans, Animals, Environments). We thank Dörte Grella and Manuel Hein (Research Center Borstel) for technical assistance.

References

1. Looney et al., Stenotrophomonas maltophilia: an emerging opportunist human pathogen. Lancet Infect Dis. 2009 May;9(5):312-23. doi: 10.1016/S1473-3099(09)70083-0. PMID: 19393961
2. Bertram et al., Status quo of tet regulation in bacteria. Microb Biotechnol. 2022 Apr;15(4):1101-1119. doi: 10.1111/1751-7915.13926. Epub 2021 Oct 29. PMID: 34713957

Proteasome inhibitors significantly increase protein levels and functionality of pathogenic pendrin variants

Florian Huber[1], Emanuele Bernardinelli[1], Raffaella Liuni[1], Lennart Weitgasser[2], Marlena Beyreis[3], Sebastian Roesch[2], Martin Jakab[3], Silvia Dossena[1]

[1]Institute of Pharmacology and Toxicology, Paracelsus Medical University, Salzburg, Austria; [2]Department of Otorhinolaryngology, Head and Neck Surgery, Paracelsus Medical University, Salzburg, Austria; [3]Institute of Physiology and Pathophysiology, Paracelsus Medical University, Salzburg, Austria; Contact: f.huber@pmu.ac.at

Objective

Pendrin is an electroneutral Cl^- / I^- / HCO_3^- exchanger. Among others, pendrin is expressed in the inner ear and the thyroid. Its main tasks in the inner ear are the maintenance of the pH and the volume of the endolymph (1,2). In the thyroid gland, an adequate production of thyroid hormones is linked to pendrin (3). Therefore, alterations in its nucleotide sequence are responsible for two of the most common forms of genetically inherited hearing loss, Pendred Syndrome (OMIM ID_274600) and non-syndromic autosomal recessive deafness B4 (DFNB4, OMIM ID_600791) (4). Both are associated with malformations in the inner ear but Pendred syndrome, in addition, leads to a thyroid dysfunction (4). Two of the most common mutations of pendrin within the Caucasian population are p.L236P and p.R409H.

Methods

HEK293 Phoenix and HeLa cells were transfected with wild type pendrin, several pathogenic variants including the L236P and R409H, or an empty vector for 48 (HEK) or 72 (HeLa) hours, followed by 6 (HEK) or 16 (HeLa) hours of incubation with a vehicle or the proteasome inhibitors MG132 or carfilzomib. Western blots and quantitative imaging were conducted on HEK and HeLa cells to determine the total protein levels, the total membrane protein levels and the plasma membrane protein levels. Functional testing was executed on HEK cells by measuring iodide influx.

Results

When the functionality of pendrin variants was decreased compared to the wild type, total, total membrane, as well as plasma membrane protein levels were significantly reduced as well. All those protein levels could be increased significantly through the treatment with MG132 or carfilzomib. Carfilzomib showed an improved ability to rescue protein levels and functionality compared to MG132.

Conclusions

The ubiquitin proteasome system is involved in the degradation of pendrin and its variants. By interfering with the ubiquitin proteasome system, the degradation of pendrin variants could be curbed. This might allow the variants to be integrated into the plasma membrane, which could ensure at least partial functionality.

Acknowledgements

This project is funded by the PMU invest in future grant (PMU-IiF_004 Dossena).

References

1. Kim HM, Wangemann P. Epithelial cell stretching and luminal acidification lead to a retarded development of stria vascularis and deafness in mice lacking pendrin. PLoS One. 2011;6(3):e17949.
2. Nakaya K, Harbidge DG, Wangemann P, Schultz BD, Green ED, Wall SM, et al. Lack of pendrin HCO3- transport elevates vestibular endolymphatic [Ca2+] by inhibition of acid-sensitive TRPV5 and TRPV6 channels. Am J Physiol Renal Physiol. 2007;292(5):F1314-21.
3. Fugazzola L, Cerutti N, Mannavola D, Vannucchi G, Beck-Peccoz P. The role of pendrin in iodide regulation. Exp Clin Endocrinol Diabetes. 2001;109(1):18-22.
4. Smith RJH, Iwasa Y, Schaefer AM. Pendred Syndrome / Nonsyndromic Enlarged Vestibular Aqueduct. In: Adam MP, Everman DB, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, et al., editors. GeneReviews(®). Seattle (WA): University of Washington, Seattle; 1993.

Galanin receptor 2/3 knockout provoked an altered immune response in a mouse peritonitis model

Sara Huber[1], Stefanie Gaisbauer[1], Antonia Gantschnigg[1,2], Andrea Ramspacher[1], Barbara Kofler[1]

[1]Research Program of Receptor Biochemistry, SALK, Salzburg, Austria; [2]Department of Surgery, Paracelsus Medical University, Salzburg, Austria; Contact: sar.huber@salk.at

Objective

A severe medical condition with inflammation of the inner abdomen wall, typically caused by infections, is called peritonitis. If not medicated timely, it can lead to life-threatening conditions. At present, immunological mechanisms driving the onset of peritonitis are still scarcely known but crucial in the development of novel therapeutic approaches. In research, sterile inflammation models are used to mimic complex chronic infections. The regulatory peptide galanin and its receptors (GAL1-3-R) are demonstrably expressed on a variety of immune cells and are key players in various diseases (1). Thus, we aimed to explore the impact of GAL2/3-R knockout (KO) on peritonitis.

Methods

Thioglycollate-elicited peritonitis was induced by intraperitoneal injection in GAL2/3-R KO and WT mice. Comparison to vehicle controls (0.9% saline) was performed to verify treatment-induced effects. 6 hours post-injection, peritoneal, spleen and blood cells were collected. Phenotyping of myeloid and T cell populations was performed via flow cytometry.

Results

Comparison of TGM-treated versus vehicle mice exhibited successful induction of acute peritonitis. During inflammation, GAL2/3-R KO mice not only revealed significantly reduced neutrophil levels in blood and spleen compared to WT mice but at the same time significantly increased Ly6C+ macrophage levels in the peritoneum. This leads to the assumption of an early initiation of adaptive immune responses at the site of inflammation, supported by the fact that dendritic cells were significantly increased in GAL2/3-R KO mice with peritonitis. T cell populations were neither affected by the treatment nor showed differences between genotypes.

Conclusions

GAL2/3-R KO mice lacked the characteristic induction of neutrophils by the innate immune system but showed induction of cells involved in early initiation of the adaptive immune responses. Future studies will be used to uncover the specific effects of single GAL2-R KO and GAL3-R KO on peritonitis. Moreover, we will investigate immune cell recruitment after 72 hours post-injection.

References

1. (1) Koller et al., Sci Rep. 2019

Exploring the potential of human iNSCs to restore connectivity in the injured spinal cord in a rat model

Felix Hübl[1,2], Lara Bieler[1,2], Katharina Günther[3,4,2], Dominika Jakubecova[1,2], Marta Suarez-Cubero[3], Christina Erhardt-Kreutzer[1,2], Ibrahim Khan[1,2], Jaqueline Illek[1,2], Thomas Weiger[5], Ludwig Aigner[4,2], Frank Edenhofer[3], Sebastien Couillard-Depres[1,2]

[1]Institute of Experimental Neuroregeneration and Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCITReCS), Paracelsus Medical University, Salzburg, Austria; [2]Austrian Cluster for Tissue Regeneration, Austria; [3]Institute of Molecular Biology and Center for Molecular Biosciences, University of Innsbruck, Innsbruck, Austria; [4]Institute of Molecular Regenerative Medicine and Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCITReCS), Paracelsus Medical University, Salzburg, Austria; [5]Department of Biosciences, University of Salzburg, Salzburg, Austria; Contact: lara.bieler@pmu.ac.at

Objective
To this day, spinal cord injury (SCI) is causing irreversible loss of function affecting all parts of the body. The current treatment approaches mainly focus on preventing progression of the injury due to secondary damages. However, a replacement of lost neuronal cells upon injury is needed to regain function. Induced neural stem cells (iNSCs), which can be directly converted from patient-specific fibroblasts, are an ideal source for cell replacement therapy and would allow autologous transplantation. Here, we investigated the potential of iNSCs transplantation in the subchronic phase of SCI in a rat contusion model.

Methods
Female Fischer-344 rats received a contusion at T8 causing moderate to severe SCI. At 30 days after contusion, fGFP-labeled human iNSCs or vehicle were injected caudal and rostral of the lesion epicenter. Due to the human origin of iNSCs immunosuppression was needed to avoid rejection of the graft. Immunosuppressive treatment was started one day before transplantation to allow natural pathophysiology within the first month of injury. Motor function was assessed at multiple time points by the BBB locomotor scale as well as the Catwalk XT system and sensory function by the Hargreave's test. Finally, after 1 month or 3 months, the cell fate of transplanted iNSCs was analyzed by immunohistochemistry.

Results
Transplantation of iNSCs at the lesion site neither improved nor deteriorated the functional outcome after SCI, compared to rats only receiving vehicle solution. Transplanted iNSCs can survive in the injured spinal cord for at least three months after transplantation according to immunohistochemistry. One month after transplantation, iNSCs mainly expressed immature NSC markers, e.g. Nestin, and early markers of neuronal differentiation, e.g. DCX and TUJ1, while glial markers were absent. In contrast, after 3 months, transplanted iNSCs exclusively expressed GFAP and neuronal as well as immature markers were absent.

Conclusions
The lesioned spinal cord constitutes an inhospitable milieu which is pro-inflammatory, full of cysts and scarred tissue and lacks proper blood supply. The survival of iNSC-derived neurons may be compromised by the environment, whereas iNSCs differentiating into astrocytes may be more robust and even contribute to the scar. Future attempts to use iNSCs to restore the neuronal network in the lesioned spinal cord should therefore address the microenvironment to improve the support, the survival and integration of iNSC-derived neurons.

Acknowledgements
This project was supported by the Wings for Life foundation.

The impact of parental mentalizing on children's mental health during COVID lockdown

Selina Ismair[1,2], Anna Krämer[3], Elisabeth Stöttinger[4], Beate Priewasser[1,2]

[1]Institute for Early Life Care, Paracelsus Medical University, Salzburg, Austria; [2]Department of Pediatrics, University Hospital Salzburg, Paracelsus Medical University, Salzburg, Austria; [3]Department of Psychology, Paris Lodron University Salzburg, Austria; [4]Hochschule Fresenius, University of applied sciences; Contact: selina.ismair@pmu.ac.at

Objective
The COVID-19 lockdown posed a significant threat to the mental health of families and children (1). One explanation for children's psychological distress could be that the additional stress that parents were exposed to led to less sensitive parenting (2). It has been suggested that mentalizing, the ability to interpret behavior by referring to inner mental states, serves as a resource against stress (3). Moreover, good mentalizing abilities in parents have a positive impact on child development (4). Indeed, studies found a positive effect of mentalizing on child outcomes during COVID-19 (5,6). However only a limited child age range and only general constructs of mentalizing have been studied. Therefore, in this study, we examine the impact of mentalizing on the link between parental stress and mental health of children of all ages during COVID-19, using the more specific construct of parental mentalizing including the inability to mentalize (prementalizing).

Methods
We surveyed 531 families, mainly mothers, with N = 871 children aged 2-18 years (M = 6.26, SD = 4.01) during COVID-19 lockdown using standardized online questionnaires to assess changes in family life, children's pediatric symptoms as an indicator of their mental health before and during the lockdown, and parental (pre-)mentalizing ability.

Results
We found a decrease in child mental health during the first lockdown, independent of child's age, which was stronger when parents were highly stressed. A moderated mediation model showed that parental stress partially mediated the link between changes in family life due to COVID-19 and increases in pediatric symptoms, and that the effect of parental stress on this increase was moderated by parental prementalization.

Conclusions
Our findings highlight the strong dependence of children's mental health on their parents, esp. in times of crisis, and emphasize the positive effect of parental mentalizing skills on child outcomes at all ages. These results inform intervention efforts to work on parental mentalizing for strengthening the whole family.

References
1. Samji H, Wu J, Ladak A, Vossen C, Stewart E, Dove N, et al. Review: Mental health impacts of the COVID-19 pandemic on children and youth – a systematic review. Child Adolesc Ment Health. 2022 May 28;27(2):173–89.
2. Cohen R, Yassin N, Gershy N. Parenting in Israel amid COVID-19: the Protective Role of Mentalization and Emotion Regulation. Advers Resil Sci. 2022 Dec 1;3(4):283–96.
3. Schwarzer, N-H, Nolte, T, Fonagy, P, & Gingelmaier, S. Self-Rated Mentalizing Mediates the Relationship Between Stress and Coping in a Non-Clinical Sample. Psychological Reports. 2022 Feb 10; 125(2): 742–762.
4. Lindblom J, Pajulo M, Nolvi S, Tervahartiala K, Karlsson H, Karlsson L and Korja R. Early pathways of maternal mentalization: Associations with child development in the FinnBrain birth cohort study. Front. Psychol. 2022 Dec 5; 13:855190.
5. Bianco F, Levante A, Petrocchi S, Lecciso F, Castelli I. Maternal Psychological Distress and Children's Internalizing/Externalizing Problems during the COVID-19 Pandemic: The Moderating Role Played by Hypermentalization. Int J Environ Res Public Health. 2021 Oct 4;18(19):10450.
6. Dollberg DG, Hanetz-Gamliel K, Levy S. COVID-19, child's behavior problems, and mother's anxiety and mentalization: A mediated moderation model. Current Psychology. 2021 Nov 9

Does fathers' attachment determine sensitivity? The mediating role of parental reflective functioning

Selina Ismair[1,2], Antonia Dinzinger[1,2], Karl Heinz Brisch[1,3], Beate Priewasser[1,2]

[1]Institute for Early Life Care, Paracelsus Medical University, Salzburg, Austria; [2]Department of Pediatrics, University Hospital Salzburg, Paracelsus Medical University, Salzburg, Austria; [3]Dr. von Hauner Children's Hospital Ludwig Maximilians University, Munich, Germany; Contact: selina.ismair@pmu.ac.at

Objective
Parental sensitivity is considered as critical factor for a healthy social-bio-psychological development of children (1) and for the formation of a secure parent-child attachment (2). In recent years, the role of fathers in child rearing has become increasingly important. Therefore, it is essential to also identify the factors contributing to father's sensitivity and extend the state of knowledge in developmental psychology, which has been primarily based on mothers.

Methods
We investigated the potential influence of attachment representations (AR) and reflective functioning (RF) on paternal sensitivity in a longitudinal design with a total of N = 40 father-child dyads. We assessed AR and RF during pregnancy using the Adult Attachment Interview (AAI) (3) as well as RF 6 months' post-partum using the Parent Development Interview (PDI) (4), both semi-structured and highly used interviews in attachment research. We coded videotaped interactions of fathers with their 6-month-old child with the Emotional Availability Scales (EAS) (5) to measure paternal sensitivity.

Results
Data analysis showed that the extent of paternal RF predicts their level of sensitivity. In combination with AR, a mediation analysis provided a more detailed explanation of the interplay between these three variables: RF fully mediated the association between AR and paternal sensitivity.

Conclusions
The homogeneity of this sample, due to high socioeconomic status and an overrepresentation of secure AR, suggests further examination of the associations with a more differentiated sample. However, this study contributes to a better understanding of the directions of action between AR, RF and sensitivity.

Acknowledgements
The first author received a PhD scholarship by Konrad Adenauer Foundation, Germany. The second author received two PhD fellowships of the Paracelsus Medical University, Austria (D-19/02/010-DIB and 2022-PRE-003-Dinzinger).

References
1. Cooke JE, Deneault A, Devereux C, Eirich R, Fearon RMP, Madigan S. Parental sensitivity and child behavioral problems: A meta-analytic review. Child Dev. 2022 Sep 31;93(5):1231 –48.
2. Verhage ML, Schuengel C, Madigan S, Fearon RMP, Oosterman M, Cassibba R, et al. Narrowing the transmission gap: A synthesis of three decades of research on intergenerational transmission of attachment. Psychol Bull. 2016 Apr;142(4):337–66.
3. George C, Kaplan N, Main M. Adult Attachment Interview . Vol. 3, Unpublished manuscript. Department of Psychology, University of California, Berkeley; 1996.
4. Aber JL, Slade A, Berger B, Bresgi I, Kaplan M. The parent development interview. Unpublished manuscript. 1985.
5. Biringen Z. Emotional Availability (EA) Scales Manual. 4th ed. emotionalavailability.com; 2008.

Type of COVID-19 vaccine influences the SARS-CoV-2 spike-protein-specific IgG antibody response

Tanja Karl[1,2], Anja Schuster[1], Lea M. Stangassinger[1], Gabriele Dobrovoljski[3], Janne Cadamuro[4], Gertie J. Oostingh[1]

[1]Biomedical Sciences, Salzburg University of Applied Sciences, Puch/Salzburg, Austria; [2]Research Program of Medical Sciences, Paracelsus Medical University, Salzburg, Austria; [3]Department of Pediatrics, Paracelsus Medical University, Salzburg, Austria; [4]Department of Laboratory Medicine, Paracelsus Medical University, Salzburg, Austria; Contact: tanja.karl@fh-salzburg.ac.at

Objective
The coronavirus disease 2019 (COVID-19) has become the single most studied human disease in history (1), putting research into high gear. There have been sustained research activities in understanding the novel acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) and new vaccines were developed at high pressure. This population based seroprevalence study focuses on the immune response following SARS-CoV-2 vaccination and/or COVID-19 by considering various influencing factors, including the influence of the type of vaccine used. Data from 2,000 Austrian subjects were collected and analyzed.

Methods
A single venous blood sample (serum, 3 ml) was taken from each individual (aged 18 and over). All participants were either vaccinated and/or COVID-19 convalescent. In order to assess the health status of the participants, a questionnaire was handed out, asking for type and date of vaccination, anthropometric data, pre-existing conditions and chronic diseases. SARS-CoV-2 spike-protein-specific IgG was then analyzed with an automated chemiluminescence immunoassay by using the VITROS® Anti-SARS-CoV-2 IgG detection method. Results are presented in binding antibody units (BAU) per ml. Statistical analysis (Kruskal Wallis test) was performed using the IBM SPSS Statistics and GraphPad Prism 9.2.0 software.

Results
Within this population study, the mean level of SARS-CoV-2-specific IgG was significantly higher ($p<0.0001$) in subjects with a hybrid SARS-CoV-2 immunity (vaccinated and convalescent) than in those with vaccination or convalescent only. In the vaccinated group, the 3 times vaccinated subjects had the highest antibody titer, which depended on the type and combination of vaccination used. Comparing the combinations three doses of Comirnaty (BNT162b2, BioNTech Manufacturing GmbH) against three doses of Spikevax (mRNA-1273, Moderna Biotech Spain, S.L.) showed a highly increased antibody titer in the Spikevax immunized population ($p<0.0001$).

Conclusions
This study highlights the differences in SARS-CoV-2 specific IgG antibody level depending on the type of vaccine as well as the combination of vaccines used. The knowledge on the effectiveness of various COVID-19 vaccines in terms of specific IgG antibody production is of great importance for future vaccine development and potential further outbreaks of new SARS-CoV-2 variants. In addition, our data supports the CDC recommendation of a third dose (booster) to retain protection, due to the significantly higher antibody level after three vaccinations.

References
1. Qi, Hai; Liu, Bo; Wang, Xinquan; Zhang, Linqi (2022): The humoral response and antibodies against SARS-CoV-2 infection. In: Nature immunology 23 (7), S. 1008–1020. DOI: 10.1038/s41590-022-01248-5.

The Mouth Breathing Diagnosis - You Do Not Know How To Diagnose A Mouth Breather

Can-Florian Keles, David Morais

[1]Prof. Dr. Rasp, PhD Medical Science, PMU, Salzburg, Austria; Contact: mouthb@protonmail.com

Objective
This educational paper discusses signs of impaired nasal respiration (mouth breathing) and its influence to the body, but investigates the diagnostic with scientific appraisal. Thereby, highlighting the importance of this inflammatory and health impacting disease. Whereby mouth breathing plays a major role in the etiology of malocclusion, negatively impacting quality of life. Finally, the paper proposes a concise scientific approach for investigations of the mouth breathing syndrome.

Methods
A concise narrative review with more than 160 citations, a guideline exceeding 25 pages.

Results
Outlook: We will describe diagnostic protocols and their benefits. We will give out recommendations for the clinician, e.g. the scientific investigator, and will display the advantages and disadvantages of each diagnostic approach.

Conclusions
 Protocols with sensors (CO2, manometer, acoustic sensor, pressure, heat detection and humidity) are proposed for objective data collection, but are time and cost intensive.
 The specialist for the nose (othorinologist) is well equipped to evaluate nasal obstructions (with the preferred technique of endoscopy). Thereby, coming very close to objectively diagnosing a subtype of chronic mouth breathers.
 The specialist for the mouth (orthodontist) can use techniques such as x-rays and special breathing tests, but is further limited in the objectiveness of diagnosing, especially acute respiratory problems.
 Questionnaires are quick to complete plus they yield valuable data as their method is quite sensitive. The NOSE (Nasal Obstruction Severity Evaluation) protocol is already a standard procedure for othorinologists, which can be used for subject comparison.

Acknowledgements
We want to thank our supervisors for all their input, our friends who proof read the texts and layout: Ricardo, Farooq Ahmed, Jassin Arnold, Tuerkan Nadire Yesil, Adith Venugopal, Bjoern Ludwig

References
1. Lin, L., et al., The impact of mouth breathing on dentofacial development: A concise review. Front Public Health, 2022. 10: p. 929165.
2. Correa, L., et al., Evaluation from the quality of life in the oral breathers patients. Arquivos Internacionais de Otorrinolaringologia, 2014. 16(01): p. 074-081.
3. Triana, B.E.G., A.H. Ali, and I.B.G. León. Mouth breathing and its relationship to some oral and medical conditions: physiopathological mechanisms involved. 2016.
4. Tresker, S., Theoretical and clinical disease and the biostatistical theory. Stud Hist Philos Biol Biomed Sci, 2020. 82: p. 101249.
5. Shastri, K., et al., Normative Values of the Nasal Obstruction Symptom Evaluation Scale. Facial Plast Surg Aesthet Med, 2023. 25(1): p. 35-39.
6. Yi, L., et al., Sagittal and Vertical Growth of the Maxillo-Mandibular Complex in Untreated Children: A Longitudinal Study on Lateral Cephalograms Derived from Cone Beam Computed Tomography. Sensors (Basel), 2021. 21(24).
7. Yu, H.-S., et al., Semi-longitudinal study of adenoid and jaw growth of normal occlusal children aged 6 to 17. Korean Journal of Orthodontics, 2000. 30: p. 699-712.

Does early Montelukast therapy improve bladder function and spinal cord regeneration after SCI?

Michael Kleindorfer[1,2], Karin Roider[1,2], Behnaz Afrashteh[1,2], Sophina Bauer[1,2], Evelyn Beyerer[1,2], Akshay Akshay[3], Katia Monastyrskaia[3], Lukas Lusuardi[2], Ludwig Aigner[1], Elena E. Keller[1,2]

[1]Institute of Molecular Regenerative Medicine, Spinal Cord Injury and Tissue Regeneration Center Salzburg, Paracelsus Medical University, Salzburg, Austria; [2]University Clinics of Urology and Andrology, Salzburg General Hospital, Salzburg, Austria; [3]Department for BioMedical Research, Functional Urology research laboratory, University of Bern, Bern, Switzerland; Contact: michael.kleindorfer@pmu.ac.at

Objective
Spinal cord injury (SCI) is one of the few medical conditions with a high unmet clinical need for emerging therapies that do not only treat the symptoms but the underlying pathology. Our research is directed towards the overshooting neuroinflammation cascade of the secondary injury which is believed to be even more destructive than the primary insult itself. With Montelukast, a licensed leukotriene receptor 1 antagonist, we hope to target one of the main elevated molecules present at the lesion site and in peripheral organs. Montelukast therapy improved non-neurogenic bladder dysfunction in patients (1) and structural bladder integrity in SCI rats (2).

Methods
Our aim was to analyze the potential of Montelukast on bladder function and spinal cord tissue after a severe T9 contusion in rats in a randomized, blinded, sham-controlled study. All animals received a permanent bladder catheter. Oral daily Montelukast therapy started on day 1 after SCI. Primary outcome parameter was the weekly functional bladder analysis, secondary outcome parameter the locomotor function. After 2 months follow up period (FUP), both spinal cord and bladder tissue, was harvested and analyzed by histology, bladder tissue additionally by transcriptomic and proteomic methods.

Results
Due to still ongoing analyses, results are shown in a blinded manner.
For all SCI rats, irrespective of group, Locomotor rating showed complete loss of function with no regain of coordinated walking. µCTs of the spinal cord confirmed consistent severe injuries. A first urodynamic pattern analysis highlighted an initial loss of bladder control with partial recovery of bladder function during week 2 and plateauing out in the second FUP month. Transcriptomics highlighted changes in proliferation, contractility and inflammation between SCI and their respective sham animals at 2 months. Animals of group A experienced more collagenisation/fibrosis, while group B had increased mitochondrial activity. Proteomic analysis found further differences between groups A and B, with B clustering closer to the healthy sham collective.

Conclusions
The potential of Montelukast will unravel once data sets are completed and de-blinded.

Acknowledgements
Supported by Wings for Life

References
1. Marschallinger J, Schäffner I, Klein B, Gelfert R, Rivera FJ, Illes S, Grassner L, Janssen M, Rotheneichner P, Schmuckermair C, Coras R, Boccazzi M, Chishty M, Lagler FB, Renic M, Bauer HC, Singewald N, Blümcke I, Bogdahn U, Couillard-Despres S, Lie DC, Abbracchio MP, Aigner L. Structural and functional rejuvenation of the aged brain by an approved anti-asthmatic drug. Nat Commun. 2015 Oct 27;6:8466. doi: 10.1038/ncomms9466. PMID: 26506265; PMCID: PMC4639806
2. Bouchelouche K, Nordling J, Hald T, Bouchelouche P. Treatment of interstitial cystitis with montelukast, a leukotriene D(4) receptor antagonist. Urology. 2001 Jun;57(6 Suppl 1):118. doi: 10.1016/s0090-4295(01)01066-4. PMID: 11378098.

The Influence of Vitamin K2 Feed of the Zucker Diabetes Fatty Rats (ZDF) on Angiogenesis using the Ex Vivo Rat Aortic Ring Assay

Maria Kokozidou[1], Clemens Gögele[1], Christian Werner[1], Gundula Schulze-Tanzil[1]

[1]Institute of Anatomy and Cell Biology, Paracelsus Medical University, Nuremberg and Salzburg, Nuremberg, Germany; Contact: maria.kokozidou@pmu.ac.at

Objective

Biological aging and chronic diseases such as diabetes mellitus or chronic kidney disease (CKD) are associated with increased incidence of cardiovascular calcification. This is accompanied by increased morbidity and mortality and is associated with atherosclerosis. A prospective cohort study showed that both vitamin (vit.) K1 and vitamin K2 supplementation was associated with a reduced risk of type 2 diabetes (T2DM). The male Zucker Diabetes Fatty (ZDF)-Leprfa/Crl rat (Fatty fa/fa and Fatty fa/+), carrying a defect leptin receptor gene is a T2DM model (Charles River 2017) induced by high protein diet. When animals are fed ad libitum with the appropriate diabetogenic diet (Purina 5008), the Fatty fa/fa rats develop T2DM at the age of about 12 weeks, while the heterozygous fa/+ rats do not become ill and are classified as control animals. The aim of this study was to distinguish the influence of vit. K2 on angiogenesis in diabetic and non-diabetic rats using an ex vivo aorta ring assay.

Methods

Adult male 10-11 weeks old animals were introduced in the experimental animal facility, PMU, Nuremberg. Animal experimental protocols were approved by the local animal human board (RUF 55.2.2-2532-2-729-17) and performed in compliance with the animal protection FELASA guidelines. Purina 5008 was administered by the supplier und further in the PMU animal facility. The animals were kept in a special incubator (55% humidity, 21+/-2°C). After at least two weeks' acclimatization time, four groups (n=3 for each group), two with ZDF fa/fa animals and two with ZDF fa/+ were formed. At 14-15 weeks old and for the next 80 days, one ZDF fa/fa and one ZDF fa/+ group were fed with Purina 5008 enriched with 100 mg/kg vit. K2 (MK-7). The other two groups were further fed with diabetogenic diet. The thoracic aorta was dissected post mortem and sectioned into 1 mm segments. 100 µl of Geltrex LDEV-free RGF basement membrane matrix were placed to solidify in each well of a 48-well plate. An aorta ring was placed in the middle of each well and incubated for 10 min at 37°C. Following, another 100 µl of Geltrex was added and solidified. Finally, 150 µl endothelial cell growth medium were added. Photographic documentation took place at time points d1, d5, d7, d9. Finally, a Live-death assay was performed. All experiments were performed in triplets.

Results

All capillary like structures remain viable until the end of the experiments. The number of the capillary like sprouts is significantly greater in the fa/fa groups compared to the fa/+ and even greater in those fed with vit. K2 at d5, d7 and d9. Similarly concerning the number of crossings that is greater in the fa/fa groups and even greater in the fa/fa animals fed with vit. K2. There is no significant difference between the groups concerning the longest sprouts. Nevertheless, there is a tendency observed that the longest sprout of each group will becomes greater in the course of the different time points.

Conclusions

T2DM is increasing the formation of the capillary like sprouts and the administration of vit. K2 enhances this effect.

Acknowledgements

This study was supported by funding of the Kerscher'sche Stiftung to MK and GST. Vitamin K2 (MK7) was kindly donated by Kappa Bioscience AS, Oslo, Norway.

References

1. Langer S, Kokozidou M, Heiiss C, Kranz J, Kessel T, Paulus N, Krüger T, Jacobs MJ, Lente C, Koeppel TA. Kidney Int. 2010; 78: 1312-1321. PMID: 20881937

Application of Pharmacophore modeling to develop new serotonine receptor 7 antagonists

Jakub Kollar[1], Sebila Pehlic[1], Daniela Schuster[1]

[1]Paracelsus Medical University; Institute of Pharmacy; Department of Pharmaceutical and Medicinal Chemistry; Strubergasse 21, 5020 Salzburg; Austria; Contact: jakub.kollar@pmu.ac.at

Objective

Serotonine is an important neurotransmitter in the human body. It mediates plethora of functions in connection with mood, sleep, pain, digestion or sexual desire.
Its effects are mediated mostly through serotonin receptors. Seven main subtypes exist, HTR1 through HTR7 (serotonine receptor 7 – 5HT7). Except for HTR3, which is ligand gated ion channel, serotonine receptors couple with G proteins (GPCR). HTR7 is coupled with Gs protein and its activation by an agonist increases the concentration of second messenger cAMP.
HTR7 receptor plays a role in development of schizophrenia, its knockout was shown to have antidepressive effects. Activation of HTR7 results in opening of TRPA1 ion channel by means of the generated second messengers. Resulting calcium influx and its increased intracellular concentration is connected with itch. In a disease like epidermolysis bullosa, where the skin is fragile and blisters easily, itching sensation might lead to scratching generating skin lesions, or more serious, deep wounds. In this project, we were focused on design of HTR7 antagonists aiming to help in itch reduction.

Methods

A set of known HTR7 antagonist and compounds inactive against HTR7 were manually collected from available literature. Five ligand-based pharmacophore models were generated and optimized to map the maximum HTR7 antagonists while not mapping any non-binders. Similarly, four homology models of HTR7 co-crystallized with antagonists were generated and structure-based HTR7 pharmacophore models were generated.

Results

Five ligand-based pharmacophore models were generated. These models were subsequently optimized to enrich the HTR7 antagonists found by these models. While models M5 and M4 are not very selective, models M1, M2 and M3 seem to be promising to suggest new potential HTR7 antagonists. M3 is the most restrictive model that maps rather small number of antagonist but no decoys. To the contrary, model M1 is rather general, identifying almost half of the antagonists, while still not mapping a large number of decoys. Based on the four HTR7 homology models, four structure based pharmacophore models for HTR7 antagonists were created.

Conclusions

The ligand-based pharmacophore models M1, M2 and M3 will be later used to screen larger databases of compounds to suggest HTR7 antagonists for biological activity testing. Database compounds mapping these models will be filtered based on their drug-like properties, similarity and toxicity and a selection will be tested in vitro on their HTR7 antagonism. The structure-based pharmacophore models need to be optimized and theoretically validated. Depending on the validation results, they might be used in the same way as here described, already optimized ligand-based models.

References

1. Tsegay EW, Demise DG, Hailu NA, Gugue ZH: Serotonin type 6 and 7 receptors as a novel therapeutic target for the treatment of Schizophrenia. Neuropsychiatric Disease and Treatment 2020. 1: 2499-2509.
2. Morita T, McClain SP, Batia LM, Pellegrino M, Wilson SR, Kienzler MA, Lyman K, Olsen ASB, Wong JF, Stucky CL, Brem RB, Bautista DM: HTR7 mediates serotonergic acute and chronic itch. Neuron 2015. 87:124-138.

VALUE-BASED HEALTHCARE FOR GLYCOGEN STORAGE DISEASE TYPE IB: REPURPOSING EMPAGLIFLOZIN

Clara Köller[1], Annieke Venema[2], Eline Bos[2], Mathieu Bolhuis[3], Enrique Contreras[4], Fred van Eenennaam[5,6], Henk Groen[7], Carla Hollak[8,9], Emma Hammerl[1], Terry Derks[2], Saskia Wortmann[1,10]

[1]University Children's Hospital, Paracelsus Medical University (PMU), Salzburg, Austria; [2]Department of Metabolic Diseases, Beatrix Children's Hospital, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands; [3]Department of Clinical Pharmacy and Pharmacology, University of Groningen, University Medical Center Groningen, The Netherlands; [4]NINA LA GUERRERA Foundation; [5]The Decision Group, Amsterdam, The Netherlands.; [6]Erasmus School of Accounting & Assurance, Erasmus University Rotterdam, The Netherlands.; [7]Department of Epidemiology, University of Groningen, Groningen, the Netherlands; [8]Department of Endocrinology and Metabolism, Amsterdam UMC, University of Amsterdam, AZ, Amsterdam, The Netherlands.; [9]Medicine for Society, Platform at Amsterdam UMC-University of Amsterdam, Amsterdam, The Netherlands.; [10]Department of Pediatrics, Amalia Children's Hospital, Nijmegen, The Netherlands; Contact: c.koeller@salk.at

Objective
Neutropenia and neutrophil dysfunction are characteristic features of Glycogen storage disease type 1b (GSD1b). Until recently, treatment involved granulocyte-colony-stimulating-factor (G-CSF) injections, which improve neutrophil count but not function. In 2019, insight into the underlying pathomechanism prompted repurposing of empagliflozin to address both neutropenia and neutrophil dysfunction.

Methods
We conducted a retrospective multicenter study including eleven subjects from the Netherlands (NL) and Austria (AT) to investigate assets of empagliflozin from a value-based health care perspective. Evaulation of personal value involved patient rated quality of life and patient reported outcome. Allocative value was assessed via medication costs, total costs including medical contacts, a sensitivity analysis and budget impact analysis. Technical value was investigated via laboratory results and (pediatric) Crohn disease activity index ((P)CDAI) and societal value via a cost evaluation and evaluation of indices for burden of disease.

Results
Empagliflozin treatment improved outcomes of all four value pillars.
Personal value: Quality-of-life scores improved by 4.5points (on a scale from one to ten) demonstrating a clear positive evolution of personal value. Allovative value: medication cost decreased by 37% (NL) and 96% (AT), total treatment costs by 47% (NL) and 66% (AT). One- and three-year economic benefits of empagliflozin likewise showed a significant cost reduction. Technical value: all patients showed clinical and laboratory improvements in neutrophil dysfunctional related findings. Eight of eleven patients showed improved and five of eleven normalized neutrophil counts, G-CSF was reduced in five and stopped in six of eleven patients. Societal value: burden of disease was reduced via a reduction in costs and required resources aswell as individual prosperity.

Conclusions
Empagliflozin is clearly superior to G-CSF from a value-based health care perspective. Empagliflozin improves laboratory outcomes, patient-reported outcomes, and is a cost-effective medical intervention. Thus, it should be established and reimbursed as first line therapy in GSD1b neutropenia and neutrophil dysfunction.

References
1. van der Zanden TM;Mooij MG;Vet NJ;Neubert A;Rascher W;Lagler FB;Male C;Grytli H;Halvorsen T;de Hoog M;de Wildt SN; Benefit-risk assessment of off-label drug use in children: The bravo framework [Internet]. Clinical pharmacology and therapeutics. U.S. National Library of Medicine; [cited 2023Apr18]. Available from: https://pubmed.ncbi.nlm.nih.gov/34145575/

ACMSD deficiency, a new disorder of tryptophan catabolism responsive to protein restriction

Clara Köller[1], René Feichtinger[1], Katja Steinbrücker[1], Ron Wevers[2], Bart Dermaut[3], Dimitri Hemelsoet[4], Martijn van Faassen[5], Ido Kema[5], Nika Schuermans[3], Johannes Mayr[1], Saskia Wortmann[1,6]

[1]University Children´s Hospital, Paracelsus Medical University, Salzburg, Austria; [2]Translational Metabolic Laboratory, Radboudumc, Nijmegen, the Netherlands; [3]Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; [4]Department of Neurology, Ghent University Hospital, Ghent, Belgium; [5]Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands; [6]Amalia Children´s Hospital, Radboudumc, Nijmegen, the Netherlands; Contact: c.koeller@gmail.com

Objective

Inborn metabolic disorders (IMDs) of amino acid metabolism can present with global developmental delay and implicate treatment options with protein restriction. The neuronal excitotoxin quinolinate, an intermediate in the de novo synthesis pathway of nicotinamide adenine dinucleotide (NAD) from tryptophan, is derived from alpha-amino-beta-carboxy-muconate-epsilon-semialdehyde (ACMS). ACMSD encodes amino-carboxymuconate semialdehyde decarboxylase, which is the only known enzyme that can process ACMS to a benign catabolite and thus prevent the accumulation of quinolinate from ACMS. Quinolinate accumulation has been related to (Alzheimer's) dementia, neuroinflammation and psychiatric disease.
Exome sequencing of a 6-year-old male with global developmental delay, autistic features and hyperactivity revealed a homozygous deletion in ACMSD involving the promotor/exon1 and predicted to lead to absence of the enzyme.

Methods

Serum quninilonate levels where meassured and psychologic testing (Kaufmann ABC) was conducted before and after dietary adaption to a low-protein (vegetarian) diet.

Results

Four-fold increased quinolinate was determined in the patient's serum. One week after implementation of a low-protein (vegetarian) diet the parents reported a clearly improved behaviour with less hyperactivity. Serum quinolinate levels were measured 4 weeks after dietary change and were comparable to those in controls.
Psychological testing (Kaufmann ABC) under normal protein load had shown a below average non-verbal IQ of 70 (85-115) with pronounced deficiencies in memory span and visual processing. Follow up testing after one year of vegetarian diet showed improvement in all investigated subareas, especially in memory span and capacity. This is also reflected by an improved non-verbal IQ of 73 (percentile ranking improved by >1).
Via matchmaking a young adult female presenting with psychiatric disease, progressive loss of skills, diffuse leukoencephalopathy on brain MRI and a homozygous truncating ACMSD variant was found. Her brain biopsy was compatible with encephalitis, further investigations are pending.

Conclusions

ACMSD deficiency is a novel IMD presenting as neurodevelopmental disorder with a spectrum encompassing developmental delay, behavioural issues to psychiatric disease with loss of skills. We have shown in one case that dietary protein restriction can limit quinolinate accumulation and seems beneficial for behaviour and possibly also learning and development.

References

1. Tryptophan metabolism [Internet]. KEGG PATHWAY: Tryptophan metabolism. [cited 2023Apr20]. Available from: https://www.genome.jp/kegg-bin/show_pathway?ec00380%2B4.1.1.45

Inhibition of cysteinyl leukotriene receptor 1 reduces levels of aggresomes and ROS in retinal pigment epithelial cells.

Andreas Koller[1], Susanne M. Brunner[1], Julia Preishuber-Pflügl[1], Daniela Mayr[1], Anja-Maria Ladek[1], Christian Runge[1], Herbert A. Reitsamer[1], Andrea Trost[1]

[1]Research Program for Experimental Ophthalmology, Department of Ophthalmology and Optometry, University Hospital of the Paracelsus Medical University, Salzburg, Austria; Contact: a.koller@salk.at

Objective
A capable endosomal-lysosomal system (ELS), which comprises cellular processes like endocytosis, trafficking and waste degradation, plays a key role in cell homeostasis. With increasing age, the ELS becomes inefficient, which leads to augmented levels of damaged organelles, reactive oxygen species (ROS) and cellular deposits, like aggresomes, and triggers the onset of age-related diseases. Consequently, the modulation of the autophagic flux, a mechanism to degrade cellular waste, seems to be a promising target to remove damaging cell waste and to treat age-related diseases, in both ocular and other tissues. Recently, we have shown that Cysteinyl leukotriene receptor 1 (CysLTR1) antagonization increases the autophagic flux in the retinal pigment epithelial cell line ARPE-19[1]. As a continuation, we investigated the effect of autophagy induction upon CysLTR1 inhibition on aggresome and ROS levels in polarized ARPE-19 cells.

Methods
The human cell line ARPE-19 was cultured and polarized under normoxic conditions. After reaching a confluence of 90-100%, cells were polarized in DMEM/F12 containing 2% FBS for 5-11 days. CysLTR1 was antagonized by using 100 nM Zafirlukast (ZK) for 3 or 24 hours. Each experiment included a time-matched vehicle (DMSO) control. Polarized ARPE-19 cells were analyzed for CysLTR1, LC3 (autophagosome/autolysosomes), LAMP1 (late endosomes/lysosomes), Proteostat (aggresomes) and Rhodamine 123 (ROS) levels using flow cytometry (FC) and immunofluorescence (IF) analysis. To determine the autophagic flux, polarized cells were additionally treated with lysosomal inhibitors and compared to controls without lysosomal inhibition.

Results
In polarized ARPE-19 cells, a subset of cells expressed CysLTR1 on the surface (SE+), which increased with polarization duration. The amount of CysLTR1 SE+ cells revealed higher levels of accumulated late endosomes/lysosomes, aggresomes and autophagosomes/autolysosomes. Interestingly, CysLTR1 SE+ cells showed an increased autophagic flux and decreased ROS formation. Furthermore, CysLTR1 inhibition for 3 hours using the antagonist ZK raised the autophagic flux. A CysLTR1 antagonizaton for 24 hours decreased ROS, late endosome/lysosome and aggresome levels in polarized CysLTR1 SE- and SE+ cells.

Conclusions
We demonstrated that CysLTR1 localization on the plasma membrane correlates with high cellular stress levels and a high autophagic activity. Whether this membrane localization of CysLTR1 may represent a novel marker to detect cellular stress or autophagy activation has to be further investigated. Furthermore, CysLTR1 inhibition probably dissembled a cell stress condition, which induces an increase of the autophagic flux and a reduction of cellular deposits in polarized ARPE-19 cells. Thus, this approach has to be transferred and tested in primary RPE cells, other CysLTR1-positive cell types and, finally, in more complex organisms to substantiate the potential of CysLTR1 antagonists as autophagy-modulating compounds for ocular aging and diseases.

References
1. Koller A. et al. Cysteinyl eukotriene receptor 1 modulates autophagic activity in retinal pigment epithelial cells. Sci Rep. 2020;10(1):17659.

Video-assisted interaction therapy for postpartum mental health problems: Effects on maternal sensitivity and competence

Katharina Kuscher[1], Sandra Gabler[2], Christine Heinisch[2], Juliane Junge-Hoffmeister[3], Susanne Simen[1]

[1]Department of Psychiatry und Psychotherapy, University Clinic of Paracelsus Medical University Nuremberg, Nuremberg, Germany; [2]Institute of Developmental Psychology, Friedrich Alexander University of Erlangen Nuremberg, Erlangen, Germany; [3]Department of Psychotherapy and Psychosomatic Medicine, Faculty of Medicine of the Technische Universität Dresden, Dresden, Germany; Contact: katharina.kuscher@klinikum-nuernberg.de

Objective
Around 10-20% of mothers suffer from mental health problems like postpartum depression and anxiety disorder after giving birth to a child (1, 2). Various mental health symptoms with postpartum onset impact the maternal interaction behavior but also the experienced competence and satisfaction as a mother (3). The present study examines the effects of video-assisted interaction therapy on maternal sensitivity, maternal competence and maternal mental health symptoms.

Methods
The study sample is part of a research-project in the Mother-Baby-Day-Unit of the Clinic of Psychiatry and Psychotherapy in Nuremberg that was conducted in cooperation with the university of Erlangen-Nuremberg. The sample consists of 78 mother-child dyads (45 mothers with postpartum mental health symptoms and 33 healthy controls). Only mothers of the clinical sample got feedback about sensitive mother-child interaction sequences. Before and after treatment symptoms of mental health, maternal sensitivity and maternal competence were assessed.

Results
Mothers with postpartum mental health problems displayed deficits in experienced maternal competence but not in observed maternal sensitivity at measuring point 1. After treatment mothers with postpartum mental health problems showed reduced symptoms of depression and anxiety. The experienced maternal competence of mothers with postpartum mental health problems increased after treatment. There was no improvement in maternal sensitivity for mothers with postpartum mental health problems after treatment.

Conclusions
Psychiatric/psychotherapeutic treatment can help to reduce symptoms of postpartum depression and anxiety. Video-assisted therapy can support enhancing maternal competence. In this preliminary analysis we found no impairment in maternal sensitivity for mothers with postpartum mental health problems. Probably the effects of postpartum mental health symptoms are stronger on internal assessment of maternal role than observable maternal behavior in interaction sequences. Ongoing analysis on potential subgroup effects will be performed.

References

1. Fawcett, E.J., Fairbrother, N., Cox, M.L., White, I.R., & Fawcett, J.M. (2019). The prevalence of anxiety disorders during pregnancy and the postpartum period: a multivariate bayesian meta-analysis. J Clin Psychiatry, 80(4): 18r12527. DOI: 10.4088/JCP.18r12527
2. O´Hara, M.W., & McCabe, J.E. (2013). Postpartum depression: current status and future directions. Annu. Rev. Psychol. (9): 379-407. DOI: 10.1146/annurev-clinpsy-050212-185612
3. Ngai, F.-W., Wai-Chi Chan, S., & Ip, Wan-Yim (2010). Predictors and correlates of maternal role competence and satisfaction. Nursing Research, 59(3): 185-193. DOI: 10.1097/NNR.0b013e3181dbb9ee

Challenges of the concept of need of nursing care in Germany – Results of a grounded theory

Miriam Laepple

[1]Institute of Nursing Science and Practice, Paracelsus Medical University, Salzburg, Austria; Contact: miriam.laepple@stud.pmu.ac.at

Objective
There are no findings on whether and how the need of nursing care is assessed by nurses in the community in Germany and to what extent the German legal definition of need of nursing care leads to challenges in the interaction between nurses in the community and people in need of nursing care.

Methods
The research question is: To what extent does the concept of need of nursing care within German ambulatory health and nursing care represent a challenge for the nurses in the community?
To address the research question, 22 problem-centred interviews were conducted with nurses in the community in Germany. The evaluation was carried out using a constructivist grounded theory according to Charmaz and Corbin/Strauss. The research methodology is based on Blumer's symbolic interactionism.

Results
Challenges exist in negotiating processes for closeness and distance, advocacy and delegation of responsibility, and ethos and technocracy. Closeness and distance are important for advocacy and ethos as fundamental elements for the balanced depth of the relationship between nurse and person in need of nursing care. Advocacy describes the social function of ambulatory nursing care: communication and coordination of care at interfaces, standing up for those in need of care when problems arise. Ethos describes a nursing orientation towards value convictions. Nurses in the community protect those in need of nursing care, e.g. from colleagues and insufficient care from their own care service. Responsibility transfer and technocracy describe actions to the contrary: Care is provided without additional commitment. Further and implicit needs of those in need of nursing care are deliberately ignored or not passed on. A technocratic attitude is closely linked to a deliberate abdication of responsibility. Need for nursing care is assessed by nurses in the community according to fixed criteria (appearance of the person and household, mobility, language and cognition, illnesses) and individual strategies (state of the toilet, existing musical instruments, interpersonal atmospheres) implicitly in a process-like manner at each home visit. The professional impression is validated or corrected by applied nursing care. Aspects such as spirituality, gender or the reflected role of the nurses themselves are not included in the assessment of the need for care.

Conclusions
The interaction-based assessment of the need for nursing care still appears to be more facility-related and is shaped by the framework conditions of the ambulatory setting in Germany. For responsibilities of social insurance law and concepts such as Community Health Nursing, it must be discussed within the profession how practical and scientific nursing can use these results for their own professionalisation and other reserved tasks.

References
1. Läpple, M. (2022). Herausforderungen des Pflegebedürftigkeitsbegriffs: Aspekte der Pflegebedürftigkeit aus Sicht von Pflegefachpersonen [Unveröffentlichte Ph.D.-Thesis]. Paracelsus Medizinische Privatuniversität, Salzburg.

Age effects on motor threshold

Christiane Licht[1], Alexandra Alario[2], Benjamin Pace[2], Anthony Purgianto[2], Mark Niciu[2], Thomas Nickl-Jockschat[2], Nicholas Trapp[2], Aaron Boes[2]

[1]Department of Psychiatry and Psychotherapy, Paracelsus Medical University, Nuremberg, Germany; [2]University of Iowa Hospitals and Clinics, Iowa City, IA; Contact: christiane.licht@klinikum-nuernberg.de

Objective
Cortical thickness and the coil-cortex distance could be affected in elderly due to the cortical atrophy occurring with age. However, previous studies were inconsistent.
We hypothesize that older patients (> 50 years) have a significantly higher resting motor threshold than younger patients (< 50 years).

Methods
This retrospective study included 129 patients with major depressive disorder (MDD) recruited from 2016 to 2020, with age from 15 – 50 years (n = 61) or from 51 – 84 (n = 68). Resting motor thresholds (RMT) were measured weekly for every patient.

Results
The older cohort of patients had a significantly (p-value: 0,001) higher average RMT of 55 % +/- 10,9 % of maximal stimulation output (1,2 T) compared to the younger patients (49 % +/- 6,9 %). Linear regression with age as a continuous variable showed a correlation coefficient of 0,36, which again underlines the apparent correlation between age and RMT.

Conclusions
These findings support the existing literature on RMT differences with age. Moreover, and with focus on elderly patients, our results are believed to contribute to tolerability differences based on age and RMT or the relationship between cortical thickness and RMT.

Acknowledgements
I was funded through the PMU travel grant. During my stay at the University in Iowa City, I conducted research in this area and collaborated with colleagues there. This research project was the result.

References
1. 1. Valiengo L, Maia A, Cotovio G, et al. Repetitive Transcranial Magnetic Stimulation for Major Depressive Disorder in Older Adults: Systematic Review and Meta-analysis. J Gerontol A Biol Sci Med Sci. 2022;77(4):851-860. doi:10.1093/gerona/glab235

Rate of volume change of chronic subdural hematomas after embolization of the middle meningeal artery

Adrian Liebert[1], Heinz Voit-Hoehne[2], Leonard Ritter[1], Thomas Eibl[1], Alexander Hammer[1], Michael Städt[2], Florian Eff[2], Markus Holtmannspötter[2], Hans-Herbert Steiner[1]

[1]Department of Neurosurgery, Paracelsus Medical University, Nuremberg, Germany; [2]Department of Neuroradiology, Paracelsus Medical University, Nuremberg, Germany; Contact: adrian.liebert@stud.pmu.ac.at

Objective
The objective of this study is to evaluate the rate of volume change of a chronic subdural hematoma after the embolization of the middle meningeal artery. This new treatment options relies on volume reduction for symptom relieve. (1)

Methods
We retrospectively analyzed the volume change of hematomas dependent on clinical and radiological factors. Volume of the hematoma before embolization and in all follow up CT scans were measured by the software Visage Imaging VISAGE 7 Version 7.1.15. Rate of volume change was then calculated as follows: rate of volume change (ml/d) = (Volume in ml in the last follow up CT scan – Volume in ml in the initial CT scan) / time differences in days between these CT scans
Volume change was measured for the whole follow-up period and within the first month.
Possible differences in the rate of volume change were then assessed in regard of factors such as the pre-interventional volume, radiological type (modified Nakaguchi Classification), embolic agent and whether the hematoma was operated before embolization or not.

Results
A total of 43 hematomas in 36 patients were treated by embolization. The mean pre-interventional volume was 68,93 ml (SD=29.59, max. 144, min 12). Most hematomas appeared homogenous hypodense (n=14) or trabecular (n=12). Other types included the homogenous iso-dense type (n=5), the homogenous hyperdense type (n=6), the gradation type (n=1) and the laminar type (n=5). The embolic agents used in this study were Onyx (n=21), Squid (n=15) and PHIL (n=7). 18 hematomas were not treated surgically prior to embolization.
Mean volume change of all hematomas was -1.95 ml/d (SD=3.55, max. 7.3, min -11.75). There was no significant influence of the pre-interventional volume (R^2=0.026, p=0.313), the radiological type (p=0.33), the embolic agent (p=0.282). There was no difference of the rate of all hematomas within the first month compared to the whole follow up period (p=0.071).
However, there was a significant faster volume change in previously surgically treated hematomas (-2.77 ml/d vs. -0.68 ml/d; p=0.046). The rate of responding hematomas without surgery within the first month was faster than the total rate (-1.44 ml/d vs. -0.94 ml/d; p=0.043).

Conclusions
Rate of volume change after embolization of the middle meningeal artery is slow with a mean rate of -1.95 ml/d in all hematomas. Previously surgically treated hematomas regress faster. Studies with a larger patient cohort and with the same follow up regime are necessary to confirm our findings.

References
1. Fiorella D., Arthur AS. Middle meningeal artery embolization for the management of chronic subdural hematoma. Journal of Neurointerventional Surgery 2019 11:912–915.

miR199a-3p INCREASES THE ANTITUMOR EFFICACY OF PALBOCICLIB IN PRECLINICAL MODELS OF HEPATOCARCINOMA

Raffaella Liuni[1,2], Elisa Callegari[2], Paola Guerriero[2,3], Cristian Bassi[2,3], Lucilla D'Abundo[2], Antonio Frassoldati[2], Edi Simoni[4], Laura Astolfi[4], Enrico Maria Silini[5], Silvia Sabbioni[3,6], Massimo Negrini[2,3]

[1]Institute of Pharmacology and Toxicology, Paracelsus Medical University, Salzburg, Austria; [2]Department of Translational Medicine, University of Ferrara, Ferrara, Italy; [3]Laboratorio Per Le Tecnologie Delle Terapie Avanzate (LTTA), Department of Translational Medicine, University of Ferrara, Ferrara, Italy; [4]Bioacoustics Research Laboratory, Department of Neurosciences, University of Padua, Padua, Italy; [5]Section of Anatomy and Pathology, University Hospital of Parma, Parma, Italy; [6]Department of Life Sciences and Biotechnology, University of Ferrara, Ferrara, Italy; Contact: raffaella.liuni@pmu.ac.at

Objective

Hepatocellular carcinoma (HCC) is the major malignant form of primary liver cancer and evolves from pre-existing chronic liver disease, particularly cirrhosis. The prognosis remains extremely poor, mainly because of the high rate of late diagnosis of the disease. Systemic therapies are the only available option for the treatment of advanced HCC. One of the drugs currently being investigated for the treatment of advanced HCC is palbociclib, a selective inhibitor of CDK4-6 that prevents the formation of the cyclinD-CDK4-6 complex and thus the phosphorylation of retinoblastoma protein (RB1), thereby blocking the cell cycle in the G1 phase. However, its antitumor effect is limited by the dose-dependent activation of AKT protein and consequently the AKT-mTOR signalling pathway, which is critical in the regulation of proliferation, survival, and chemoresistance Hence the hypothesis of increasing the antitumor efficacy of palbociclib by combining it with the AKT inhibitor miR199a-3p. [1]

Methods

miR199a-3p expression was induced in the human HCC cell lines HepG2 and Hep3B by using adeno-associated virus. Quantitative assessment of cell viability and cell counts were performed by using the Muse Count & Viability kit with the Muse Cell Analyser instrument. The Muse Annexin V & Dead Cell Assay kit was used for quantitative analysis of living, apoptotic or dead cells. Western Blots were performed for molecular analysis and quantification of protein levels of AKT, RB1, and their phosphorylated forms. The effect of the combination of palbociclib and miR199a-3p was evaluated in vivo in the TG221 transgenic mouse model, which is characterized by predisposition to liver tumor development. Therapy lasted 21 days after tumor development. To ensure miRNA expression, a strategy of miRNA mimics delivered by nanoparticles administered by intraperitoneal injection every 3 days was exploited. The occurrence and volume of nodules was monitored by ultrasonography.

Results

The combination of palbociclib with miRNA induced a greater proapoptotic and antiproliferative effect compared with palbociclib or miR199a-3p alone in HepG2 and Hep3B cells. Also in vivo, miR199a-3p in combination with palbociclib proved more effective than the single drugs and increased the antitumor effect by reduction of tumor nodules without development of toxicity.

Conclusions

These results lay the foundation for further preclinical investigations and also suggest a new therapeutic strategy for the treatment of advanced HCC. Micro RNA-based molecules in combination with known drugs might improve their antitumor efficacy while maintaining a good safety profile.

References

1. Callegari E, D'Abundo L, Guerriero P, Simioni C, Elamin BK, Russo M, et al. miR-199a-3p Modulates MTOR and PAK4 Pathways and Inhibits Tumor Growth in a Hepatocellular Carcinoma Transgenic Mouse Model. Molecular therapy Nucleic acids. 2018;11:485-93

Capsulorhexis with Ultrashort Laser Pulses in the Picosecond Regime in the cataract surgery, a dream becoming reality.

Francesco Luciani[1], Josef Schmidbauer[1], Michael Körber[2], Bernd Braun[2], Manfred Kottcke[2], Andreas Giese[2], Milan Fritsche[2], Jonathan Wenk[2], Daniel Kopf[3]

[1]Augenklinik - Klinikum Nord Nürnberg.; [2]Technische Hochschule Nürnberg; [3]Montfort Laser GmbH; Contact: Francesco.Luciani@klinikum-nuernberg.de

Objective

The goal of the project is to perform a continuous curvilinear capsulorhexis (CCC) by using picosecond ultrashort pulse (USP) lasers in the cataract surgery. We developed a new laser source in the Picosecond regime and tested it on pig eyes as animal model.

Methods

After coupling the laser beam with a conventional slit lamp, we performed incisions of the anterior capsule of pig eyes. We used a USP laser source to emit pulses with 12 ps pulse width at 1064 nm. We compared the incisions through picosecond laser to those through nanosecond laser.

Results

Due to the very short pulse widths of USP lasers, microprocessing of tissues can be performed with reduced pulse energy. As a result, only a very small amount of heat is deposited in the tissue with each pulse and the shock waves are greatly reduced. This prevents the tissue from evaporating and tearing, and results in a far more precise and controllable tissue ablation. For this reason, the process is also known as cold material removal.

Performing a continuous curvilinear capsulorhexis (CCC) on animal model through our USP laser source was possible and lead to high quality incisions.

Tissue ablation is far more precise and deterministic with a USP laser than with a nanosecond laser source.

Conclusions

Our results showed the feasibility of performing high quality continuous curvilinear capsulorhexis (CCC) through USP lasers on animal models.

The pig eye is so similar to the human eye that we can very easily speculate that the same technology would lead to excellent clinical results during cataract surgery on patients.

Acknowledgements

This project has received funding from the Eurostars-2 joint programme with co-funding from the European Union Horizon 2020 reasearch and innovation programme.

References

1. Vogel, A., Capon, M. R., Asiyo-Vogel, M. N., and Birngruber, R., “Intraocular photodisruption with picosec_x0002_ond and nanosecond laser pulses: Tissue effects in cornea, lens, and retina,” Investigative Ophthalmology and Visual Science 35(7), 3032–3044 (1994).
2. Vogel, A., Busch, S., Jungnickel, K., and Birngruber, R., \Mechanisms of intraocular photodisruption with picosecond and nanosecond laser pulses," Lasers in Surgery and Medicine 15(1), 32{43 (1994).
3. Friedman, N. J., Palanker, D. V., Schuele, G., Andersen, D., Marcellino, G., Seibel, B. S., Batlle, J., Feliz, R., Talamo, J. H., Blumenkranz, M. S., and Culbertson, W. W., \Femtosecond laser capsulotomy," Journal of Cataract and Refractive Surgery 37(7), 1189{1198 (2011).
4. Liang, X.-X., Linz, N., Freidank, S., Paltauf, G., and Vogel, A., \Comprehensive analysis of spherical bubble oscillations and shock wave emission in laser-induced cavitation," Journal of Fluid Mechanics 940, A5 (jun 2022).

Immunosuppressive function of AML-EVs on adaptive and innate immune cells

Nicole Maeding[1], Heide-Marie Binder[1], Balasz Vari[1], Anna Raninger[1], Andre Cronemberger Andrade[1], Thomas Heuser[2], Astrid Obermayr[3], Gabriele Brachtl[1], Lisa Pleyer[4,5], Richard Greil[4,5], Katharina Schallmoser[6], Dirk Strunk[1]

[1]Experimental and Clinical Cell Therapy Institute (ExCT), Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TreCS), Paracelsus Medical University, Salzburg, Austria; [2]Vienna Biocenter Core Facilities, Medical University, Vienna,Austria; [3]Department of Cell Biology, Biomedical Ultrastructure Research Group, University of Salzburg, Salzburg,Austria; [4]3rd Medical Dept. with Hematology, Medical Oncology, Rheumatology and Infectiology, Paracelsus Medical University, Salzburg,Austria; [5]Salzburg Cancer Research Institute (SCRI), Center for Clinical Cancer and Immunology Trial (CCCIT) and Cancer Cluster Salzburg (CCS), Salzburg,Austria; [6]Department of Transfusion Medicine, Paracelsus Medical Private University, Salzburg, Austria; Contact: nicole.maeding@pmu.ac.at

Objective

Extracellular vesicles (EV) are enriched in acute myeloid leukemia (AML) patient's blood and were found to contribute to migration, invasion and chemoresistance of AML cells. Although under intense investigation, the mechanisms by which AML-derived EVs contribute to the immunosuppressive microenvironment remain poorly understood. We and others have recently published that EV functions are mediated in part by factors within a protein corona around EVs[1]. Here we asked if immune functions of AML-EVs depend on corona-associated factors.

Methods

AML-derived EVs from conditioned medium were enriched >100x using two sequential rounds of tangential flow filtration (TFF), separating AML-derived soluble factors from EVs in parallel. AML patient plasma-derived EVs were isolated by size exclusion chromatography. Purified AML-derived EVs were used as active agent in assays interrogating T cell proliferation and NK cell-dependent cytotoxicity. To determine the contribution of corona-associated factors, EVs were further purified by ultracentrifugation resulting in corona-depleted core EVs (TUCF) and corona-associated factors (solF).

Results

Purified EVs showed a significant dose-dependent inhibition of PHA-stimulated T cell proliferation. Furthermore, cell-line and primary patient-derived AML-EVs were able to reduce NK cell-mediated lysis of target cells. EV corona-associated factors (solF) were responsible for inhibition of cytotoxicity as corona-depleted EVs did not show an inhibitory effect. In contrast, modulation of PHA-stimulated T cell proliferation was independent of corona factors.

Conclusions

We show that AML-EVs inhibit T cell proliferation and NK cell functionality with differential contributions of EV corona- and core-associated factors. These findings highlight target-dependent modes of action of AML-EVs and could aid in implementing novel strategies for specific AML-EVs-based therapeutic interventions.

Acknowledgements

This work received funding from the European Union's Horizon 2020 research and innovation program and by Land Salzburg in the framework of EV-TT, EV-TT - Bpro

References

1. 1. Wolf M, Poupardin RW, Ebner-Peking P, Andrade AC, Blöchl C, Obermayer A, Gomes FG, Vari B, Maeding N, Eminger E, Binder HM, Raninger AM, Hochmann S, Brachtl G, Spittler A, Heuser T, Ofir R, Huber CG, Aberman Z, Schallmoser K, Volk HD, Strunk D. A functional corona around extracellular vesicles enhances angiogenesis, skin regeneration and immunomodulation. J Extracell Vesicles. 2022 Apr;11(4):e12207. doi: 10.1002/jev2.12207. PMID: 35398993; PMCID: PMC8994701.

The Cdk-5 inhibitor Dinaciclib as a novel approach in biliary tract cancer.

Theresa Mähr[1], Petra Huber-Cantonati[1], Celina Ablinger[1], Christian Mayr[2], Tobias Kiesslich[2], Markus Ritter[2], Johanna Pachmayr[1]

[1]Institute of Pharmacy, Department of Pharmaceutical Biology and Clinical Pharmacy, Paracelsus Medical University, Salzburg, Austria; [2]Center of Physiology, Pathophysiology and Biophysics, Institute of Physiology and Pathophysiology, Paracelsus Medical University, Salzburg, Austria; Contact: theresa.maehr@stud.pmu.ac.at

Objective
Biliary tract cancer (BTC), which includes cholangiocarcinoma (CCC), is a rare and aggressive type of malignant cancer that originates in the epithelial cells of the bile duct. The incidence of CCC varies greatly by geographic region. In Western countries, CCC accounts for about 3% of all gastrointestinal malignancies. The prognosis for patients with CCC is poor due to the lack of available effective treatment options. Surgical resection remains the most promising curative treatment, although this is not possible in an advanced stage diagnosis. Other treatment options include chemotherapy, radiotherapy and liver transplantation for patients diagnosed at an early stage of the disease. Due to the lack of an effective therapy for late diagnosed cholangiocarcinoma, a new therapeutic approach is urgently needed. The aim of this study was to evaluate the anti-proliferative effect of the Cdk-5 inhibitor Dinaciclib in 3D spheroids of BTC cells and furthermore the combination of Dinaciclib with Cisplatin.

Methods
To investigate the effect of Dinaciclib on BTC cells, a proliferation assay was performed in 3D spheroids. This was done by seeding different amounts of cells into each well of a 96-well ULC ultra-low attachment plate. After an initial growth period of three days, the spheroids were stimulated with the compounds for 96 hours. Spheroid formation and size were monitored and imaged before and after treatment using an Olympus CKX53SF microscope. Cell viability after treatment was measured using Promega CellTiter-Glo® 3D cell viability assay. This assay determines the number of viable cells in a 3D cell culture by quantifying the cells' ATP level, which is a marker for the presence of metabolically active cells.

Results
All cell lines except HuCCT-1 show significantly decreasing cell viability with increasing concentration of Dinaciclib. The most significant effect of Dinaciclib on the cell viability is observed in MMNK-1 and NOZ. Moreover, CCC-5 cells are resistant to low concentrations of Cisplatin. However, above 25 µM Cisplatin reduces the cell viability significantly. Furthermore, combination of Dinaciclib and Cisplatin declines the cell viability in 3D spheroids compared to the control and mono-treatments, although no significant synergistic effect could be shown.

Conclusions
Dinaciclib shows a promising effect as a novel therapeutic approach in BTC by significantly reducing cell viability of 3D spheroids. This study has provided the basis for further experiments. The combination of Dinaciclib and Cisplatin showed no synergistic effect at the concentrations used. However, this should not be ruled out as a possibility, but should be pursued further in other variants and settings. The results shown support the promising potential of Dinaciclib in BTC therapy.

Acknowledgements
This project is funded by PMU-FFF research fund (E-19/30/157-ARD).

References
1. Valle JW, Kelley RK, Nervi B, Oh DY, Zhu AX. Biliary tract cancer. Lancet. 2021;397(10272):428-44.

Intraoperative hemoadsorption lowers need for inotropic support and reduces in-hospital mortality in Staphylococcus Aureus cohort in active left-sided infective endocarditis.

Hazem Mamdooh[1], Jurij Matija Kalisnik[1,2], Zibert Janez[3], Leiler Spela[1], Bagaev Erik[1], Fittkau Matthias[1], Fischlein Theodor[1]

[1]Department of cardiac surgery, Nuernberg hospital, Paracelsus Medical University, Nuernberg, Germany; [2]Medical School, University of Ljubljana, Ljubljana, Slovenia; [3]Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia; Contact: hazemmamdooh@yahoo.com

Objective
Sepsis by infective endocarditis (IE) is associated with significant mortality (1). Blood purification using hemoadsorption (HA) may attenuate the inflammatory response. We investigated the effect of intraoperative HA on postoperative outcomes in active left-sided all-comers and staphylococcus aureus (StaphA) IE cohort.

Methods
Prospectively collected 181 variables from consecutive patients with IE were retrieved in a single-center retrospective study between 01/2015 and 05/2022. Patients with intraoperative HA were compared to patients without HA in all-comers and StaphA pts. Sequential Organ Failure Assessment (SOFA) score was assessed daily. The primary outcome was vasoactive inotropic score (VIS), secondary outcome was in-hospital mortality.

Results
Baseline characteristics were similar in all-comers (265 pts.) and in StaphA cohort (68 pts.), except for more history of stroke in HA all-comers, 40 (15.1%) vs. 17 (8.7%), p=0.033. Intraoperative HA received 135 (51%) all-comers and 30 (44%) StaphA pts. Similar VIS score was observed at all times in all-comers, lower VIS score was found consistently in the HA-group with 15.0 [4.8;25.2] vs. 28.5 [15.1;51.7], p=0.017; 5.8 [3.6;23.9] vs. 27.0 [5.9;55.4], p=0.014 and 5.0 [0.0;17.0] vs. 17.1 [4.8;44.1], p=0.017 at 8h, 16h and 24h in StaphA cohort. In-hospital mortality was similar in all-comers with 24 (9.1%) vs. 27 (10.2%), p=0.644 but significantly lower in HA-group of StaphA pts. with 3 (10.0%) vs. 17 (44.7%), p=0.03. Intraoperative HA was associated with odds ratio (OR) 0.83 [95%CI:0.45-1.52], p=0.537 in all-comers and OR 0.14 [95%CI:0.04-0.53], p=0.004 in StaphA pts. The SOFA score on the second postoperative day was the strongest independent predictor of in-hospital mortality in all-comers (OR 1.21 [95%CI:1.07-1.36]), p=0.003, as well as in StaphA pts. (OR 1.46 [95%CI:1.04-2.04]), p=0.029.

Conclusions
Adjunctive intraoperative hemoadsorption was associated with lower need for inotropic support on the first postoperative day and reduced in-hospital mortality in StaphA cohort but not in all-comers with active left-sided IE.

References

1. Pettersson, G.B.; Hussain, S.T. Current AATS guidelines on surgical treatment of infective endocarditis. Ann. Cardiothorac. Surg. 2019, 8, 630–644.

Glymphatic system in ocular diseases – MRI findings

Panagiota Manava[1], Francesco Luciani[2], Josef Schmidbauer[2], Michael M. Lell[1], Klaus Detmar[1]

[1]Department of Radiology and Nuclear Medicine, Klinikum Nuernberg, Paracelsus Medical University, Germany; [2]Department of Ophthalmology and Visual Science Klinikum Nuernberg, Paracelsus Medical University, Germany; Contact: panagiota.manava@klinikum-nuernberg.de

Objective
The diffusion of Gadolinium (Gd) in the Glymphatic system through the blood-brain-barrier (BBB), the Liquor Cerebrospinalis (CSF)-barrier and blood ocular barrier has been recently demonstrated, especially in delayed MRI imaging with a signal increase in different CSF compartments (the anterior eye compartment, the Meckel's cave, the suprasellar cistern, the internal auditory canal and the ambient cistern). These findings support the hypothesis, that Gd diffuses into the CSF through the choroid plexus, since it is being leakier than the BBB and the aqueous chamber of the eye. In this study we want to investigate contrast dynamics in different eye compartments associated with specific ophthalmologic diseases in MRI Scans.

Methods
In 95 patients (63 with ophthalmological disease, 32 control group) specific MRI scans on a 1.5-T MRI unit were acquired. Three scans were performed, first as baseline before intravenous contrast application of Gd and two after with a delay of 20 and 120 minutes after contrast application.
Patients were divided according to their location of pathology in anterior (AEC) and posterior eye compartment (PEC). Relative signal intensity (SI) increase in anterior eye chamber (AC), vitreous body with retina (VB), optic nerve sheath (ONS) and Meckel's cave (MC) was analyzed and correlated with ophthalmologic diseases.

Results
In patients with a disorder in AEC significant SI increase was found in central AC (p20min/= 0.000, p120min= 0.000), lateral AC (p20min = 0.001, p120min =0.005) and VB (p20min = 0.02) compared to the control group; those with pathologies in PEC showed higher SI levels in the central AC (p120min =0.041) and VB (p120min =0.006).

Conclusions
Increased Gd enhancement was found in central and lateral AC and VB in patients with AEC, suggesting an impairment of the blood-aqueous barrier. In patients with a disorder in PEC, pathological enhancement indicates a disruption of the blood-retinal-barrier allowing Gd to diffuse into VB from posterior to anterior opposite to the known physiological pathway of the glymphatic system.

References
1. Manava P, Eckrich C, Luciani F, Schmidbauer J, Lell MM, Detmar K. Glymphatic System in Ocular Diseases: Evaluation of MRI Findings. AJNR Am J Neuroradiol. 2022 Jul;43(7):1012-1017. doi: 10.3174/ajnr.A7552. Epub 2022 Jun 30. PMID: 35772805; PMCID: PMC9262072.

Out-patient Treatment of Internet Gaming Disorder among Adolescence

Philipp Martzog[1], Melanie Sczudlek[1], Patrick Nonell[1]

[1]Kinder- und Jugendpsychiatrie/Klinikum Nürnberg; Contact: philipp.martzog@klinikum-nuernberg.de

Objective
Internet gaming disorder (IGT) among adolescents is associated with personal and social impairments and has become an important public concern[1]. Reviews of interventions studies provide evidence for cognitive behavioral therapy and psychopharmacotherapy in treatment of IGT[2]. However, considering the target groups typically high resistance to participate in hospital treatment, low-threshold interventions are needed. The few existing outpatient treatment programs however fall short in supporting adolescence to regain control over screen-time through effectively substituting the latter by alternative non-digital activities[3,4]. The current study aims at the formative evaluation of an adaptation of the outpatient intervention „Lebenslust statt Onlineflucht“[3]. Specifically, it is evaluated if combining traditional cognitive behavioral therapy with individual coaching adolescents in the acquisition of action-control strategies and sportsskills across a 10-weeks treatment program will reduce symptoms of IGT and help participants to become more self-determined.

Methods
The current study uses a one-group, pretest/posttest/follow-up design with an intended sample of 25 adolescence (age range 12-18) in an out-patient psychiatric facility. The three measurement points will be 3 months apart, resulting in a 9-month overall study-interval. The sample will encompass participants diagnosed with symptoms of IGD (gaming and/or social-media) using both, standardized objective and subjective assessment tools. Objective measures include app-based assessments of screen-time activities and amount of movement and sports activities. Likely psychiatric comorbidities will be captured using the Child Behavior Checklist (CBCL/6-18R). Self-report measures encompass GADIS-P+A, CSAS and SOMEDIS.

Results
Data from the current study will be analyzed using repeated measures ANOVA. Expected results should reveal decreases in screen-time and IGD-symptoms among participants across measurement points on the one hand and a corresponding increase in sports-related activities on the other hand.

Conclusions
Results will be used to further improve the adapted version of the treatment program „Lebenslust statt Onlineflucht“[3]. In particular, further information will be provided on the role of individual coaching in goal-striving, action-control and sports skills among adolescence.

Acknowledgements
The project and research receive financial support through the Emanuel Wöhrl Foundation.

References
1. Paschke K, Holtmann M, Melchers P, Klein M, Schimansky G, Krömer T et al. Medienbezogene Störungen im Kindes- und Jugendalter: Evidenzpapier der Gemeinsamen Suchtkommission der kinder- und jugendpsychiatrischen und psychotherapeutischen Fachgesellschaft und Verbände (DGKJP, BAG, BKJPP). Z Kinder Jugendpsychiatr Psychother 2020; 48(4):303–17.
2. Chang C-H, Chang Y-C, Yang L, Tzang R-F. The Comparative Efficacy of Treatments for Children and Young Adults with Internet Addiction/Internet Gaming Disorder: An Updated Meta-Analysis. Int J Environ Res Public Health 2022; 19(5). 10.3390/ijerph19052612
3. Moll B, Thomasius R. Kognitiv-verhaltenstherapeutisches Gruppenprogramm für Jugendliche mit abhängigem Computer-oder Internetgebrauch: das" Lebenslust statt Onlineflucht"-Programm. Göttingen: Hogrefe Verlag GmbH & Company KG; 2019.
4. Ily D, Florack J. Behandlungsmanual Videospiel- und Internetabhängigkeit: Verhaltenstherapeutisch-orientierte Gruppenbehandlung zur Teilabstinenz bei Adoleszenten: das "Git Gud in Real-Life"-Programm. 1. Auflage. München: Elsevier; 2021.

Prognostic factors on clinical outcome in patients with epidural hematoma

Petra Mercea[1,2], Iain Walker[2], Bradley Harrington[2], Zahier Ebrahimi[2], Adriaan Vlok[2]

[1]Department of Neurosurgery, Paracelsus Medical University, Salzburg, Austria; [2]Department of Neurosurgery, Stellenbosch University, Cape Town, South Africa; Contact: p.mercea@salk.at

Objective

Management of traumatic brain injuries (TBI) with epidural hematomas (EDH) depends on early diagnosis and, if indicated, neurosurgical evacuation.[1] Prognostic factors have rarely been investigated in large studies and the influence of time from injury to surgery on patients' outcome has been discussed controversially.[2] Our aim was to perform a systematic analysis of a large patient cohort to assess factors associated with outcome.

Methods

Adult patients with EDH admitted to Tygerberg Hospital, South Africa, between January 2011 and February 2022 were included. Severity of head injury was classified as mild (GCS 13-15), moderate (GCS 9-12) and severe TBI (GCS 3-8). Outcome was graded based on Glasgow Outcome Scale Extended (GOSE) at discharge and dichotomized into unfavorable (GOSE 1-5) and favorable (GOSE 6-8).

Results

We included 223 patients of whom 115 patients (52%) had mild, 47 (21%) moderate, and 61 (27%) severe TBI (Table 1). We found a clear correlation between TBI severity, motor score on admission, pupillary status, and age with outcome. The mechanism of injury, size of midline shift, and EDH volume were not associated with unfavorable GOSE. Neither did time from injury or admission to surgery influence patients' outcome. However, clinical severity of TBI, EDH volume and midlineshift had a clear influence on the timing of surgery.

Conclusions

Severe TBI has a devastating impact on patients' prognosis. This study shows that clinical characteristics such as GCS at admission, pupillary status and age were predictive factors of outcome and had an impact on how fast patients were operated. This is in accordance with the current guidelines on EDH. Nevertheless, the authors suggest further analyses on these prognostic factors to investigate the indications for surgery and thus future management of patients with EDH.

References

1. Rosyidi R, Priyanto B, Al Fauzi A, Sutiono A. Toward zero mortality in acute epidural hematoma: A review in 268 cases problems and challenges in the developing country. Interdiscip Neurosurg. 2019 Sep 1;17:12–8.
2. Leitgeb J, Mauritz W, Brazinova A, Majdan M, Wilbacher I. Outcome after severe brain trauma associated with epidural hematoma. Arch Orthop Trauma Surg. 2013 Feb];133(2):199–207.

Project Mouth Breathing (PMB)

David Morais, Can-Florian Keles

[1]Prof. Dr. Rasp, PhD Medical Science, PMU, Salzburg, Austria; Contact: mouthb@protonmail.com

Objective
PMB is set to be an explorative investigation into the prevalence (prospective and controlled cross-sectional study) of several altered variables in MB patients. With a focus on a descriptive picture of temporomandibular disorder (TMD) and tongue evaluation as well as their sleep score in a home setting, where there is no current research.

Methods
Orthodontic patients presenting themselves in the OralKlass clinics in Porto, Portugal will be asked to participate in the breathing study. The sample size calculation results suggest at least 10 versus 10 subjects to achieve the desired power (alpha: 5%; beta: 10%, confidence interval: 90%).
They will be screened for MB / nose breathing (control group) by an othorinologist (using endoscopy to grade the obstruction level). Followed by multiple measurement assessments (oxygen saturation with an oximeter, blood pressure is measured, orthodontic analyses of x-rays/pictures/casts & clinically for IOTN-classification/angle measurements/tooth and jaw positioning, posture, questionnaires (Ribeiro and NOSE) will be tested, TMD assessment - using the DC/TMD protocol, oral hygiene classified by plaque-index and bleeding index as well as color assessment and sleep-score evaluations by the home use of the SnoreLab App).

Results
Current status The first challenge we encountered is, that there is actually no gold standard for a MB-diagnosis. At the moment we are in the process of publishing a methodological paper on the diagnoses.

Conclusions
The causes of orthodontics malalignment (etiology) is basic research. With the withdrawal of public funds in this area it has been underinvestigated. Our research will contribute to understanding of the effects of mouth breathing. The current scientific evidence level in this field is low.

Acknowledgements
We want to thank our supervisors for all their input, our friends who proof read the texts and layout: Ricardo, Farooq Ahmed, Jassin Arnold, Tuerkan Nadire Yesil, Adith Venugopal, Bjoern Ludwig

References
1. Schiffman, E., et al., Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: recommendations of the International RDC/TMD Consortium Network* and Orofacial Pain Special Interest Groupdagger. J Oral Facial Pain Headache, 2014. 28(1): p. 6-27.

AGING OF THE VASCULAR NICHE IN ALZHEIMER'S DISEASE - ASSOCIATION WITH STRING VESSELS

Heike Mrowetz[1,2], Kathrin Kniewallner[1,2], Michael Unger[1,2], Diana Bessa de Sousa[1,2], Ludwig Aigner[1,2,3]

[1]Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg, Austria; [2]Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University, Salzburg, Austria;; [3]Austrian Cluster for Tissue Regeneration; Contact: heike.mrowetz@pmu.ac.at

Objective
Around 60-90% of Alzheimer's disease (AD) patients show cerebrovascular pathological alterations linked to neurodegeneration, including vascular Aβ accumulation, fragmented blood vessels, changes in blood vessel diameter and thickness of the vascular basement membrane of capillaries (reviewed in [1-4]). Cerebrovascular dysfunction is also linked to a compromised blood-brain barrier (BBB) structure and function. However, the molecular and cellular changes leading to BBB dysfunction remain largely unexplored. Here, we aim to characterize vascular and cellular changes occurring at the neurovascular unit in a mouse model of AD and aged mice.

Methods
In a quantitative immunohistochemical approach, we used collagen IV and PDGF receptor beta staining to assess vessel density, volume and fragmentation, as well as pericyte number and pericyte coverage of blood vessel, in brain sections from 3-, 10- and 12-months old in APP-PS1 mice and wild type (WT) littermates.

Results
We observed a decrease in vessel density and vessel volume as well as an increase vessel fragmentation with aging. In aged APP-PS1 mice, these vascular alterations were more pronounced than in aged WT mice. We also observed an increase in string vessels (i.e., avascular basement membrane strands, remnants of capillaries) in APP-PS1 mice already at pre-plaque stages. Pericyte numbers were highly decreased in aged APP-PS1 and WT mice.

Conclusions
Age-related cellular and structural changes of neurovascular unit appear earlier and are more pronounced in AD transgenic mice, potentially contributing to the disease progression.

References
1. Selkoe, D., Alzheimer's disease: Conversation with an expert. Harv Health Lett, 2002. 27(11): p. 5.
2. Selkoe, D.J., Alzheimer's disease: genes, proteins, and therapy. Physiol Rev, 2001. 81(2): p. 741-66.
3. Selkoe, D.J., Alzheimer's disease is a synaptic failure. Science, 2002. 298(5594): p. 789-791.
4. Selkoe, D.J. and J. Hardy, The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med, 2016. 8(6): p. 595-608.

A protocol to evaluate and validate implant internal forces and moments

Mischa Muehling[1,2], Sabrina Sandriesser[1,2], Peter Augat[1,2]

[1]Institute for Biomechanics, BG Unfallklinik Murnau, Germany; [2]Institute for Biomechanics, Paracelsus Medical University Salzburg, Austria; Contact: mischa.muehling@bgu-murnau.de

Objective

A wide variety of implant types are used to address several challenges of fracture fixation. Manufacturers develop new models in order to face different fracture scenarios or improve the existing implants. There is limited knowledge about the internal forces and moments acting on the implants while implanted in the human body. Therefore, new implants are commonly tested and approved with respect to their corresponding predecessor products, because the mechanical requirements are unclear. The aim of this study is to evaluate and validate implant internal forces and moments and translate the complex physiological loading case into a simplified 3 or 4 point bending loading case of the implant.

Methods

A finite elements model for a transverse femur shaft fracture (AO/OTA type 32-B2) treated with a locked plate system (AxSOS 3 Ti Waisted Compression Plate Broad, Stryker, Kalamazoo, USA) was developed and experimentally validated. The fractured construct was physiologically loaded by resulting forces on the hip joint from previously measured in-vivo loading experiments (Bergmann et. al). The forces were reduced to a level where the material response in the construct remained linear elastic. Resulting forces, moments and stresses in the implant of the fractured model were analysed and compared to the manufacturers' approval data.

Results

The FE-model accurately predicted the behaviour of the whole construct and the micro motion of the working length of the osteosynthesis. The resulting moment reaction in the working length was 24 Nm at a load of 400 N on the hip. The maximum principle strains on the locking plate were predicted well and did not exceed 1 %.

Conclusions

In this study we presented a protocol by the example of locked plated femur shaft fracture to evaluate and validate implant internal forces and moments. It included calculation of implant internal loading using finite element analysis and validation of the internal loading. The presented protocol can be used to identify the internal loads of several different implants. The obtained information about the physiological situation of implants can be used to optimize the design of the implants.

References

1. AO Surgery Reference: https://surgeryreference.aofoundation.org/orthopedic-trauma/adult-trauma/femoral-shaft/segmental-intact-middle-1-3-fractures/mio-bridge-plating 30.08.2022
2. Bergmann G, Bender A, Dymke J, Duda G, Damm P (2016) Standardized Loads Acting in Hip Implants. PLOS ONE 11(5): e0155612. https://doi.org/10.1371/journal.pone.0155612

Scar-in-a-jar: Establishment of an in vitro assay to study fibrotic scaring in spinal cord injury

Alina Munschke[1,2], Sébastien Couillard-Després[1,2,3], Lara Bieler[1,2,3]

[1]Institute for Experimental Neuroregeneration, Paracelsus Medical University, Salzburg, Austria; [2]Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Austria; [3]Austrian Cluster of Tissue Regeneration; Contact: alina.munschke@stud.pmu.ac.at

Objective
A spinal cord lesion is caused by two mechanisms, beginning with primary injury mechanisms due to initial mechanical damage. This is followed by secondary injury mechanisms due to a series of cellular, molecular and biochemical processes. The process culminates in a glial and fibrotic scar. Understanding scar formation and its visualization in vitro is an essential foundation for my study.
The Scar-in-a-Jar is based on the principle of an in vitro fibrosis model. The model includes a faster collagen biosynthesis with complete deposition despite a short culture time. This work will first prove the concept of scar-in-a-jar using primary dermal adult human fibroblasts (HDFa). We then aim to establish and validate the model for primary adult rat fibroblasts from the dura mater of the spinal cord (RSDF).

Methods
We applied the protocol for the Scar-in-a-Jar by Stebler and Raghunath (2021). The conditions tested were, on the one hand, the control medium with and without transforming growth factor beta 1 (TGF-β1). On the other hand, we tested macromolecular crowding (MMC) medium with and without TGF-β1. Fluorescent and picrosirius red staining were used for the best possible comparison of the cell lines. Furthermore, the picrosirius red staining allowed an additional determination of collagen content by absorbance measurements. Finally, we performed paired t-tests to determine statistical differences between conditions.

Results
The amount of collagen production of HDFa confirmed that HDFa is a collagen-producing cell type as described in the literature. The treatment conditions did not significantly affect the collagen production of HDFa. Only the time factor showed that TGF-β1 has a positive effect on collagen production. In RSDF, the use of TGF-β1 significantly increased the collagen production. However, this effect was only detectable when normalizing the measurements to the cell count. The morphological appearance of the cell lines differs in control conditions. Especially upon TGF-β1 treatment the morphology of the RSDF changes, while the morphology of the HDFa remained unchanged. MMC is only having a small impact on the morphology of the cells. In both cell lines, collagen was mainly located in the endoplasmic reticulum. Collagen was also found outside the cells.

Conclusions
The Scar-in-a-Jar model provides a good basis for studying collagen formation and consequently the development of scars. However, our results show how differently RSDF in comparision th HDFa behave in the experiment and how variable the influences of MMC and TGF-β1 can be. Still, we have shown that the RSDF produce collagen 1 in our culture system. The next step would be a validation using antifibrotic substances.
Additionally, further research is needed with, for example, other cell types such as astrocytes and pericytes which are known to contribute to scar formation in spinal cord injury. In this way, the complexity and process of scar formation in spinal cord injury could be better captured in vitro.

References
1. STEBLER, S. & RAGHUNATH, M. 2021. The Scar-in-a-Jar: In Vitro Fibrosis Model for Anti-Fibrotic Drug Testing. In: Hinz, B., Lagares, D. (eds) Myofibroblasts. Methods in Molecular Biology, vol 2299. Humana, New York.

Beyond T cell Toxicity – Intrathecal CXCL13 elevation indicates B-cell involvement in neurological immune-related adverse events following immune checkpoint inhibition in two oncology patients

Ferdinand Otto[1], Lara Bieler[1], Michael Seiberl[1], Waltraud Kleindienst[1], Peter Kölblinger[2], Andrea Harrer[1,2]

[1]Department of Neurology, Christian-Doppler University Hospital, Paracelsus Medical University, Centre for Cognitive Neuroscience, Member of EpiCARE, Salzburg, Austria; [2]Department of Dermatology and Allergology, Paracelsus Medical University, Salzburg, Austria; Contact: f.otto@salk.at

Objective
Neurological immune-related adverse events (nirAE) occur in 1-5% following treatment with immune checkpoint inhibitors (ICI). Mainly attributed to autoreactive T-cells, nirAE usually respond to steroids. Severe outcomes are rare and reported in clinically overlapping syndromes with accumulating evidence of B cell autoimmunity.

Methods
We present two patients who developed grade 3-4 nirAE with evidence suggestive for B cell involvement.

Results
CASE 1: A 75y female with metastatic melanoma developed double vision and general weakness 2 weeks after the 1st cycle ICI therapy with ipilimumab and nivolumab. Workup showed pleocytosis (27 cells/µl) with elevated CXCL13 (316 pg/ml) in cerebrospinal fluid (CSF). Increased creatine kinase, troponin T, elevated levels of anti-acetylcholine receptor antibodies and positive anti-titin antibodies led to the diagnosis of myocarditis and myositis/myasthenia gravis overlap syndrome. The patient received high-dose steroids resulting in normalization of cell count and CXCL13 in CSF but persistent elevation of cardiac enzymes despite normal cardiac output in serial echocardiography. Flow cytometry revealed intrathecal recruitment of CXCL13-responsive B cell helper T cells of a predominant inflammatory Th1 and Th17 phenotype and increased CD4/CD8 T cell ratio of 18.5. Non-responding to steroids, the patient received additional therapy with immunoglobulins (IVIG). Further escalation with rituximab could not be realized because the patient rapidly deteriorated and deceased.
CASE 2: A 75y female with metastatic lung adenocarcinoma developed ataxic gait and ataxia 5 months after chemotherapy combined with ICI (pembrolizumab). CSF showed marked pleocytosis (248 cells/µl), CXCL13 elevation and positive anti-neuronal cerebellar antibodies, suggesting ICI-mediated cerebellitis. The patient received high-dose steroids and IVIG for 5 days each, clinically improved, was discharged but readmitted 5 months later due to subacute onset of spastic paraparesis. Magnetic resonance imaging of the spine revealed thoracic myelopathy and CSF showed mild pleocytosis (5 cells/µl) and mild CXCL13 elevation. A second cycle of steroids was followed by rituximab and achieved clincal stability.

Conclusions
We describe two cases with intrathecal CXCL13 elevations, which highlight uncertainties in diagnosis and treatment of ICI-associated nirAE. CXCL13 may serve as diagnostic tool for B cell involvement. Early treatment escalation with B cell depleting agents such as rituximab may help prevent fatal clinical courses.

Acknowledgements
This work was partly supported by the PMU-FFF grant R-18/01/100-HAP.

References
1. Yin B et al. Myocarditis and myositis/myasthenia gravis overlap syndrome induced by immune checkpoint inhibitor followed by esophageal hiatal hernia: A case report and review of the literature. Front. Med 9:95080, 2022, PMID: 36457566

SGLT2 inhibitors reduce cytosolic calcium transient in a diabetic cardiomyopathy rat model with preserved ejection fraction

Vera Paar[1], Peter Jirak[1], Fabian Föttinger[1], Bernd Minnich[2], Falk Schrödl[3], Andrea Trost[4], Andreas Koller[4], Michael Lichtenauer[1], Uta C. Hoppe[1], Lukas J. Motloch[1]

[1]Division of Cardiology, Department of Internal Medicine II, Paracelsus Medical University, Salzburg, Austria; [2]Division of Vascular and Exercise Biology, Department of Biosciences, University of Salzburg, Salzburg, Austria; [3]Institute for Anatomy, Paracelsus Medical University, Salzburg, Austria; [4]University Clinic of Ophthalmology and Optometry, Research Program for Experimental Ophthalmology and Glaucoma Research, Paracelsus Medical University Salzburg, Salzburg, Austria; Contact: v.paar@salk.at

Objective

Dapagliflozin (DAPA), a highly potent selective sodium-glucose cotransporter 2 (SGLT2) inhibitor, is widely used for the treatment of type 2 diabetes (T2D) (1). Recent data unveiled beneficial effects of SGLT2 inhibitors on cardiovascular events in heart failure with preserved ejection fraction (HFpEF), including diabetic cardiomyopathy. Nevertheless, described pathophysiological mechanism of beneficial effects are still a matter of debate. Concerning this, experimental models in rodents revealed modulatory effects of DAPA on cardiac calcium (Ca^{2+}) handling in diabetes (2), though its effect in diabetic cardiomyopathy cardiomyocytes is rather speculative.

Methods

To confirm diabetic cardiomyopathy, echocardiography was performed in the diabetic cardiomyopathy rat model of Otsuka Long-Evans Tokushima Fatty (OLETF) rats at baseline (18 weeks) and at the age of 36 weeks. Intracellular and mitochondrial Ca^{2+} currents were analyzed in isolated ventricular cardiomyocytes from OLETF rats at the age of 36 weeks. Upon continuous contraction in a field stimulation chamber, the fluorescent signals (Fluo-4, AM and Rhod-2, AM) of DAPA treated and untreated cells were recorded at 37°C.

Results

Echocardiography indicated a significant decrease but still preserved ejection fraction (EF) in our rat model (EF>80%). Importantly, an increase in IVSs (19.0 mm, IQR = 19.0-20.0 mm; $p<0.0001$) and IVSd (14.0 mm, IQR = 14.0-15.0 mm; $p<0.0001$) as well as biatriale enlargement (right atrium: 30.4 mm, IQR = 30.0-31.0, $p<0.0001$; left atrium: 37.0 mm, IQR = 35.0-38.0 mm, $p<0.0001$) were observed after 36 weeks, indicating diastolic dysfunction. Interestingly, in 36 weeks rats, Ca^{2+} transient measurements unveiled a significant reduction of intracellular Ca^{2+} currents when treated with 10 µM DAPA ($p=0.0009$), while mitochondrial Ca^{2+} currents were not influenced by the DAPA treatment ($p=0.1543$).

Conclusions

In our model of cardiomyopathy with preserved ejection, DAPA treatment seems to reduce cytosolic but not mitochondrial Ca^{2+} transient. These results indicate major alterations of Ca2+ handling in diabetic cardiomyocytes. Further, studies need to investigate the effect of SGLT-2 inhibitors on the cardiomyocytes' function and electrophysiological properties.

Acknowledgements

This study was supported by Paracelsus Medical University (R-018/02/105/-JIR).

References

1. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med. 2019 Jan 24;380(4):347-357. doi: 10.1056/NEJMoa1812389. Epub 2018 Nov 10. PMID: 30415602.
2. Hamouda NN, Sydorenko V, Qureshi MA, Alkaabi JM, Oz M, Howarth FC. Dapagliflozin reduces the amplitude of shortening and Ca(2+) transient in ventricular myocytes from streptozotocin-induced diabetic rats. Mol Cell Biochem. 2015 Feb;400(1-2):57-68. doi: 10.1007/s11010-014-2262-5. Epub 2014 Oct 29. PMID: 25351341.

Multi-Stakeholder Design for Complex Digital Health Systems: Development of a Modular Open Research Platform (MORE)

Pavithren V S Pakianathan[1], Daniela Wurhofer[1], Devender Kumar[1], Josef Niebauer[1,2], Jan Smeddinck[1]

[1]Ludwig Boltzmann Institute for Digital Health and Prevention; [2]University Institute of Sports Medicine, Prevention and Rehabilitation, Paracelsus Medical University, Salzburg, Austria; Contact: pavithren.pakianathan@dhp.lbg.ac.at

Objective
To implement an iterative development approach integrating multi-stakeholder perspectives to support a single platform development process.

Methods
Capture, fulfil and balance the requirements of a multi-disciplinary group of stakeholders interacting with the system through a Delphi-inspired, iterative and participatory design process encompassing a series of workshops and online surveys.

Results
Through interaction with a multi-disciplinary group of key platform stakeholders, diverse feedback and requirements for the design and development process were elicited and integrated.

Conclusions
Findings from the initial rounds of stakeholder involvement lay the stepping stone towards further iterations in the process. Experts who participated in the process reported being generally supportive of and feeling involved in the development process.

Acknowledgements
This project is funded by: Science and Innovation Strategy Salzburg 2025 (WISS 2025) of the state of Salzburg.

References
1. D. Kumar, S. Jeuris, J. E. Bardram, and N. Dragoni, “Mobile and Wearable Sensing Frameworks for mHealth Studies and Applications: A Systematic Review,” ACM Trans. Comput. Healthc., vol. 2, no. 1, p. 8:1-8:28, Dec. 2021, doi: 10.1145/3422158.
2. R. J. Widmer, N. M. Collins, C. S. Collins, C. P. West, L. O. Lerman, and A. Lerman, “Digital Health Interventions for the Prevention of Cardiovascular Disease: A Systematic Review and Meta-analysis,” Mayo Clin. Proc., vol. 90, no. 4, pp. 469–480, Apr. 2015, doi: 10.1016/j.mayocp.2014.12.026.
3. S. Beck et al., “The Open Innovation in Science research field: a collaborative conceptualisation approach,” Ind. Innov., vol. 29, no. 2, pp. 136–185, 2022.
4. I. Nahum-Shani et al., “Just-in-Time Adaptive Interventions (JITAIs) in Mobile Health: Key Components and Design Principles for Ongoing Health Behavior Support,” Ann. Behav. Med., vol. 52, no. 6, pp. 446–462, May 2018, doi: 10.1007/s12160-016-9830-8.
5. D. Barrett and R. Heale, “What are Delphi studies?,” Evid. Based Nurs., vol. 23, no. 3, pp. 68–69, Jul. 2020, doi: 10.1136/ebnurs-2020-103303.

The Relationship between Glucose and the Liver-Alpha Cell Axis – A Systematic Review

Thomas Pixner[1,2], Nathalie Stummer[2,3], Anna Maria Schneider[2,3], Andreas Lukas[1,2], Karin Gramlinger[1], Valérie Julian[4], David Thivel[5], Katharina Mörwald[2,3], Harald Mangge[6], Christopher Dalus[2,3], Elmar Aigner[2,7], Dieter Furthner[1,2], Daniel Weghuber[2,3], Katharina Maruszczak[2,3]

[1]Department of Pediatric and Adolescent Medicine, Salzkammergutklinikum Voecklabruck, 4840 Voecklabruck, Austria; [2]Obesity Research Unit, Paracelsus Medical University, 5020 Salzburg, Austria; [3]Department of Pediatrics, Paracelsus Medical University, 5020 Salzburg, Austria; [4]Department of Sport Medicine and Functional Explorations, Diet and Musculoskeletal Health Team, Human Nutrition Research Center (CRNH), INRA, University Hospital of Clermont-Ferrand, University of Clermont Auvergne, 63000 Clermont-Ferrand, France; [5]Laboratory of Metabolic Adaptations to Exercise under Physiological and Pathological Conditions (AME2P), University of Clermont Auvergne, 63000 Clermont-Ferrand, France; [6]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, 8010 Graz, Austria; [7]First Department of Medicine, Paracelsus Medical University, Salzburg, Austria; Contact: tom.pixner@gmx.at

Objective
Until recently, glucagon was considered a mere antagonist to insulin, protecting the body from hypoglycemia. This notion changed with the discovery of the liver-alpha cell axis (LACA) as a feedback loop. The LACA describes how glucagon secretion and pancreatic alpha cell proliferation are stimulated by circulating aminoacids (AAs). Glucagon in turn leads to an upregulation of amino acid metabolism and ureagenesis in the liver. Several increasingly common diseases (e.g., non-alcoholic fatty liver disease, type 2 diabetes (T2D), obesity) disrupt this feedback loop [1]. There is limited knowledge of glucagon and amino acid dynamics after glucose administration, hence the purpose of this systematic review was to summarize what is known on the interactions between glucose and the LACA.

Methods
The authors of this systematic review applied PRISMA guidelines and conducted PubMed searches to provide results of 8078 articles. The abstracts were screened and if they appeared relevant, studied in full.

Results
In total 88 articles were included in this review. Secondary literature from these articles was also analyzed and referenced. Nineteen articles included standardized glucose administration (i.e., oral glucose tolerance test (OGTT) or intravenous (i.v.) glucose). One article investigated both glucagon and AAs. Twelve articles examined the effect of glucose administration on glucagon, six articles studied the effect of an OGTT on AAs. Six articles included pediatric subjects.

Conclusions
The LACA is still understudied. Certain results (e.g., inverse association between glucagon and glucose in youth vs. adults, the change of AA-levels with age) hint towards differences between adults and children concerning LACA and pathophysiology. While hyperglycemia might directly attenuate alpha cell function, the consequences for the LACA remain unclear. Impaired glucagon response during OGTT is present in patients with T2D, but also in healthy individuals. The results on AAs in the fasting state remain partly inconclusive, but branched-chain amino acids are associated with metabolic impairments and show different dynamics in an altered LACA. The LACA may provide better insight into metabolic diseases, and the dynamics of glucagon and amino acids during standardized glucose challenge tests may hold a predictive or diagnostic value.

References
1. Wewer Albrechtsen NJ, Færch K, Jensen TM, et al. Evidence of a liver-alpha cell axis in humans: hepatic insulin resistance attenuates relationship between fasting plasma glucagon and glucagonotropic amino acids. Diabetologia. 2018;61(3):671-680.

Analysis of the extracellular vesicle research field over the past decade: Cargo and function correlate with isolation method.

Rodolphe Poupardin[1], Martin Wolf[1], Liliia Paniushkina[1,2], Sven Geissler[3], Katharina Schallmoser[4], Gregor Fuhrmann[5], Kenneth W Witwer[2], Dirk Strunk[1]

[1]Cell Therapy Institute, Paracelsus Medical University, Salzburg, Austria ; [2]Depts. of Molecular and Comparative Pathobiology and Neurology, Johns Hopkins University School of Medicine, Baltimore, USA; [3]BIH Center for Regenerative Therapies (BCRT), Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117, Berlin, Germany ; [4]Institute of Transfusion Medicine, Paracelsus Medical University, Salzburg, Austria; [5]Department of Biology, Friedrich-Alexander-University Erlangen Nuremberg, Erlangen, Germany; Contact: rodolphe.poupardin@pmu.ac.at

Objective
The evolution of extracellular vesicle (EV) research enabled fascinating discoveries by introducing nanotechnology into biomedical cell communication science. Here, we conducted an unbiased study to illustrate the evolution of EV isolation methods, phenotype, cargo, and function analysis over the last decade.

Methods
We performed a text mining-based systematic analysis of 20,364 EV-related records identified in PubMed 2013–2022.

Results
We found EV isolation significantly correlating with cargo and function, pointing to potential relationship between EV manufacture and applicability. Blood/plasma/serum, urine and cell culture media were most-analyzed EV sources. Studies combining 2-4 EV isolation, characterization or functionality methods significantly increased over time. Among 2,386 papers reporting particle counts, just 156 (6.5%) reported normalized particle-protein ratio.

Conclusions
Based on this study, we generated a searchable open-source database as up-to-date information resource. We further created an online checklist (www.myevcheck.org) enabling standardized methods and results reporting to facilitate research and development. Checklist completion produces a downloadable automatically formatted PDF providing an overview for scientists, technology developers, journal editors, reviewers and readers in this fast-changing field.

References
1. J. Lötvall, et al, JEV. 2014; https://doi.org/10.3402/jev.v3.26913.
2. C. Théryet al., JEV. 2018; https://doi.org/10.1080/20013078.2018.1535750.
3. R. Poupardin et al., Adv Drug Del Rev 2021; https://doi: 10.1016/j.addr.2021.113872
4. M. Wolf et al., JEV 2022; https://onlinelibrary.wiley.com/doi/full/10.1002/jev2.12207

Effects of probiotic supplementation in preterm infants - preliminary results of a QI project

F. Präuner[1], N. Rochow[1], K. Knab[1], S. Hofmann[2], K. Benkert[2], M. Selivanov[2], J. Gillich[2], R. Ebbert[2], G. Torbahn[1], S. Schäfer[1], J. Zimmermann[1], S. Lohmüller-Weiß[1], A. Szakacs-Fusch[1], A. Meis[1], K. Simon[1], C. Fusch[1]

[1]Department for neonates, children and adolescents, Paracelsus Medical University, Nuremberg, Germany; [2]Faculty of applied chemistry, Technische Hochschule Nürnberg, Nuremberg, Germany; Contact: franziska.praeuner@stud.pmu.ac.at

Objective
Randomized trials have described an association between reduction of NEC, sepsis and mortality and administration of probiotics in more than 10000 preterm infants (PI). However, the effects of composition (species), dosage, duration of the microbiome are not well understood. ESPGHAN (1) recommends the combination of Bifidobacterium infantis (subspecies of B. longum), B. lactis (subspecies of B. animalis), and Streptococcus (S) thermophilus. As part of a quality improvement initiative (QI), we have established the administration of these recommended probiotics in our routine practice. The aim of this study is to evaluate the microbiome in treated and non-treated infants.

Methods
PI with a gestational age (GA) <32 weeks or birth weight (bw) <1500g, with minimum of ≥50ml/kg/d of enteral intake, received ProPrems® (Neobiomics, Sweden; dose: 2x 0.5g/d equivalent to 2x 0.5 billion/d. units) within 120 hours of life. Breast-fed PI received only a "starter" dose for 7 days and formula-fed PI received the dose continuously until 34 weeks PMA. Antibiotic therapy resulted in interruption of probiotic administration with restart after discontinuation. Stool samples were collected weekly and DNA sequencing was performed with Oxford Nanopore Technologies to identify prokaryotic 16S-rDNA and classified using QIIME2TM. Analysis of taxonomic data was performed using ANCOM.

Results
N=140 (GA: 29, 23-41 (median, range), bw: 1,410±670g) were included. Of these, n=48 received breast milk (BM)+formula, n=52 BM, and n=40 formula. n=93 received antibiotics. N=33 infants of the control group (GA: 34, 30-36 (median, range), bw: 2260±430) participated. Of these, n=12 received BM+formula, n=7 BM, and n=14 formula. n=11 received antibiotics. To date, 259 stool samples were analyzed. 14 subjects of the control provided 44 samples. During the intervention, DNA of the probiotics was detected in almost all stool samples. After the interv., DNA from B. longum was still found in 91% of the samples. B. animalis was only detectable in 9% and S. thermophilus in 25%. In the control, B. animalis was not found at all, S. thermophilus was only found in 14% and B. longum in 25% of the samples. Comparison of species frequencies showed significant differences for S. thermophilus and B. animalis during the interv. and all other groups (pre-, post- and control group). For B. longum, samples during and after the interv. were not significantly different; however, these two groups differed significantly from the samples before interv. and the control. The percentage of B. longum in the stool was high (Median of reads Mor=1277.5) and remained so after the interv. (Mor=2184). B. animalis had only Mor=7 and S. thermophilus Mor=24. Both were only rarely detectable after the interv..

Conclusions
ProPrems supplementation resulted in appearance of the species in almost all stool samples, with only B. longum as a significant proportion of the stool microbiome. This was unexpected, since the three species were given at the same dosage. That only B. longum remained stable after the end of the interv., while B. animalis and S. thermophilus were undetectable in many samples, refutes the assumption that breast milk prebiotics sustain the probiotics used. Future studies should investigate probiotic dose and neonatal outcome.

References
1. van den Akker CHP, van Goudoever JB, Shamir R, Domellöf M, Embleton ND, Hojsak I, et al. Probiotics and Preterm Infants: A Position Paper by the European Society for Paediatric Gastroenterology Hepatology and Nutrition Committee on Nutrition and the European Society for Paediatric Gastroenterology Hepatology and Nutrition Working Group for Probiotics and Prebiotics. J Pediatr Gastroenterol Nutr. 2020 May;70(5):664–80.

Specific Medical Simulation Trainings for Parents of MPS Patients – An Innovative Approach and its Psychological Benefits

Florian Lagler[1], Beate Priewasser[2], Christina Lampe[3], Carmen Kunkel[4], Michaela Weigl[5], Marc Baumann[6], Anna-Maria Wiesinger[1]

[1]Institute of Congenital Metabolic Diseases, Paracelsus Medical University, Salzburg, Austria; [2]Institute of Early Life Care, Paracelsus Medical University, Salzburg, Austria; [3]Department of Child and Adolescent Medicine, Center of Rare Diseases, University Hospitals Giessen/Marburg, Giessen, Germany; [4]Gesellschaft für Mukopolysaccharidosen e.V., Aschaffenburg, Germany; [5]MPS-Austria, Gesellschaft für Mukopolysaccharidosen und ähnliche Erkrankungen, Scharten, Austria; [6]Medical Simulation Center, Paracelsus Medical University, Salzburg, Austria; Contact: florian.lagler@pmu.ac.at

Objective
MPS patients have a substantial risk for respiratory, cardiac and other emergencies. Therefore, we train physicians, nurses and recently parents of MPS patients within medical simulation trainings (MST). In MPS specific MST for more >600 nurses and physicians we observed improvements of patient safety relevant competencies comparable to MST in other target groups. The parental trainings however, seem to have relevant psychological effects, which are analyzed within this inter-professional project.

Methods
Motivated by perceived psychological benefits in parents, we conducted a quasi-experimental controlled study with a pre-post comparison and a follow-up assessment of psychological wellbeing, anxiety (STAI), recovery and stress (PSQ, EBF), self-efficacy (SWE) and family quality of life (FLQ).

Results
After 4 of 8 planned MST, the preliminary results of our online survey from currently 62 participants (82% parents, 16% other family members and 2% professionals) indicates that parents of children with an increased medical risk for emergencies (n = 12), show significantly higher levels of fear and stress load. Participants of previous MST (n = 23) showed less situational anxiety (p = .052) and experienced stress (p = .007) as compared to MST naive individuals.

Conclusions
The preliminary results of our study indicate that fears of emergencies and psychological stress are substantial in MPS parents and further highlight, that MST indeed have a positive impact on the quality of life of affected caregivers and families. We expect to provide sufficient evidence with this study to establish MST as a relevant and rational part of high-quality healthcare in MPS and other rare chronically debilitating metabolic disorders. We aim to present the final study results by the end of this year.

References
1. Walker R, Belani KG, Braunlin EA, et al. Anaesthesia and airway management in mucopolysaccharidosis. J Inherit Metab Dis. 2013;36(2):211-219. doi:10.1007/s10545-012-9563-1
2. Kuratsubo I, Suzuki Y, Orii KO, Kato T, Orii T, Kondo N. Psychological status of patients with mucopolysaccharidosis type II and their parents. Pediatr Int. 2009;51(1):41-47. doi:10.1111/j.1442-200X.2008.02652.x
3. Raluy-Callado M, Chen WH, Whiteman DA, Fang J, Wiklund I. The impact of Hunter syndrome (mucopolysaccharidosis type II) on health-related quality of life. Orphanet J Rare Dis. 2013;8:101. Published 2013 Jul 10. doi:10.1186/1750-1172-8-101

Impact of selective S1P receptor modulation on vaccination-induced SARS-CoV-2 reactive T cell response in multiple sclerosis

Richard Friedrich RADLBERGER[1], Thomas HAYBÄCK[1], Ferdinand OTTO[1], Michael SEIBERL[1], Lara BIELER[1], Tobias MOSER[1], Georg PILZ[1], Andrea HARRER[1,2], Peter WIPFLER[1]

[1]Department of Neurology, Christian Doppler Clinic, Paracelsus Medical University, Salzburg, Austria; [2]Department of Dermatology and Allergology, Paracelsus Medical University, Salzburg, Austria; Contact: dr.radlberger@gmx.net

Objective
The treatment approach for MS is changing to “early intense therapy” strategy in hope to maximize the clinical benefits in the long-term. Opposing this, are concerns about the immune competence especially in times of a pandemic like SARS-CoV-2. As shown in recent studies sphingosine 1-phosphate receptor (S1P) modulators attenuate the serologic response to SARS-CoV-2 vaccination and infection.
Further evidence is needed to understand whether S1P receptor modulators, particularly the selective ones, also influence T cell responses. Therefore, we are investigating the specific vaccine-induced SARS-CoV-2 T cell response after therapy induction with ozanimod, a selective S1P1 and S1P5 receptor modulator.

Methods
We included 10 MS patients (80% female) assigned to selective S1P receptor modulation with ozanimod and six age and gender-matched normal controls. All but two (20%) patients had at least two vaccinations prior to treatment start. SARS-CoV-2 reactive T cells were measured before (baseline, T0) treatment initiation and after three (T3M) and six (T6M) months in patients, and three (T1) and six (T2) months after the third vaccination in normal controls using the SARS-CoV-2 ProtS Complete T Cell Analysis Kit (PBMC9 from Miltenyi Biotec (Bergisch Gladbach, Nordrhein-Westfalen, Germany). Cells were analyzed by flow cytometry and CD4+ and CD8+ SARS-CoV-2 reactive T cells were defined by intracellular accumulation of TNF-α and/or IFN-γ.

Results
In ozanimod-treated patients, SARS-CoV-2 reactivity was evident both in CD4+ and in CD8+ T cells. There was a clear reduction in SARS-CoV-2 reactivity of CD4+ T cells over time but not in CD8+ T cells. Normal controls showed an increase in CD4+ T cells, which most likely was due to break-through infections in 4/6 controls between the two measurements. Of note, breakthrough infections occurred also in 4/10 patients who showed a trend to a slighter decrease in SARS CoV-2 reactivity than those without break through infection.

Conclusions
Our real-life study about SARS-CoV-2 reactivity in the recall revealed to be more complex, as we did not expect such high frequency of break-through disease in patients and controls after full vaccination. This made the interpretation of the data difficult.
However, in summary, we can conclude that SARS-CoV-2 reactive T cell responses remain detectable during the first 6 months of treatment with ozanimod. The CD4+ but not CD8+ T cell response show a decline. Breakthrough infections despite vaccination occurred in our cohort, but were neither more frequent nor more severe than in normal controls.

Acknowledgements
This study was supported by Bristol Myers Squibb. The authors directed and are fully responsible for all content and editorial decisions for this abstract.

Measuring infant's movements in clinical routine using either _x000B_electro-magnetic or optical tracking systems

Ferdinand Rehme[1,2], Maximilian Tiedemann[1,2], Christoph Fusch[1,3], Niels Rochow[1,3,4]

[1]Department of Peditrics, Paracelsus Medical University, Nuremberg, Germany; [2]Nuremberg Institute of Technology, Nuremberg, Germany; [3]Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada; [4]Department of Pediatrics, University Medicine Rostock, Rostock, Germany; Contact: rehmefe78651@th-nuernberg.de

Objective

Adequate nutrition and an individual adjusted energy intake are crucial for physical and neurological development of infants. One reason for this is the predestination of children for obesity and cardiovascular diseases. Inadequate nutrition may lead to reduced muscle development and a weakened immune system. However, excessive energy intake can lead to unfavorable fat deposition and expedition of chronic diseases. The aim of this study is therefore to measure the spontaneous movement of preterm infants within warming beds or incubators with the future goal to calculate the required energy intake. Additionally, it might be possible to measure neurological development out of the individual movement data.

Methods

Newborns from the Nuremberg clinic were included in the study if parental consent was available. Body movement was measured using the NDI 3D Guidance trakSTAR electro-magnetic tracking system with six sensors, applied to the head, chest, hands and feet of the infants. Due to major accuracy issues within the interference field of the warming beds, experiments with the Intel D435 3D camera have been made in the second step. Here, the mediapipe (v 0.7) pose-detection algorithm and the pyrealsense2 library (Intel® RealSense™ SDK 2.0) have been used.

Results

Measurements with the electro-magnetic system in the laboratory environment and simple movements patterns have been successful, after the raw data has been filtered. Simple circular motions with a circumference of 52 cm could be made over 45 times with an error of less than 1.2%. The unfiltered error for this measurment is 9.6%. However, the more complex movement patterns of infants and the significant interference noise led to wrong results of parallel measured movements on the station. These parallel measured movements were measurements, that have been additionally tracked by hand and compared afterwards. This showed that the unfiltered measurements could be shorter or longer than the actual distance for the same sensor on different movements, therefor having no clear systematic error in the clinical environment. Nevertheless, sleeping patients showing near to no movement at all were correctly tracked. For instance, a patient that was at a near standstill for fifteen minutes, had an unfiltered overall distance of 8.9 m and a filtered overall distance of 0.24 m. It has been shown so far that the camera can recognize and track the model of a child. In addition, the real-world coordinates of the head, chest, hands and feet can be calculated.

Conclusions

Measurements with the NDI 3D Guidance trakSTAR haven been too unreliable in the clinical setup to measure the fine spontaneous movements of infants, especially over a long period of time. The measurements with the optical system are more promising, but still have to prove themselves in a clinical setting. It may be necessary to filter the raw movement data here as well, should the pose detection algorithm become too inaccurate for premature infants.

Measurement of umbilical vessel lengths on x-ray images for the development of umbilical catheters

Ferdinand Rehme[1,2], Naghmeh Forghani[2], Stefan Eckstein[4], Stanimir Georgiev[5], Peter Ewert[5], Ravi Selvaganpathy[6], Salhab el Helou[3], Christoph Fusch[1,3], Niels Rochow[1,3,7]

[1]Department of Peditrics, Paracelsus Medical University, Nuremberg, Germany; [2]Nuremberg Institute of Technology, Nuremberg, Germany; [3]Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada; [4]Eckstein Design, Munich, Germany; [5]Department of Pediatric Cardiology and Congenital Heart Defects, German Heart Center Munich, Technische Universität München, Munich, Germany; [6]Department of Mechanical Engineering, McMaster University, Hamilton, Ontario, Canada; [7]Department of Pediatrics, University Medicine Rostock, Rostock, Germany; Contact: rehmefe78651@th-nuernberg.de

Objective

A considerable number of preterm and term infants suffer from respiratory and renal insufficiency during postnatal adaptation. The application of an artificial placenta requires large bore vessel access. The catheter position for the artificial placenta is different compared with standard umbilical catheter. The umbilical artery catheter should be positioned at the junction umbilical artery-internal iliac artery. The umbilical vein should be positioned at the level of the top of diaphragm. Anatomical data needed to design umbilical catheters for the artificial placenta and 3D models of umbilical vessels for simulation are scarce. The aim of this study is to measure the anatomical path of umbilical vessels of preterm and term infants for different weight groups and to establish a 3D data set.

Methods

In this study five weight strata were defined for body weight (<800g; 800g to 1,249g; 1,250g to 1,999g; 2,000g to 2,999g; ≥3,000g) including five infants each. Infants with umbilical vein catheter and umbilical artery catheter displayed in anteroposterior and lateral view of chest and abdominal X-ray were included. The dimensions of the umbilical vessels were measured using IC Measure (The Imaging Source Europe GmbH, v 2.0.0.286) and a Python (Python Software Foundation, v 3.11.1) script. To measure the length of the vessel, individual nodes were placed over the course of the inserted catheter on the provided radiographs. The nodes could then be exported, scaled to real-world-coordinates and the total distance measured. The total distances of the placed umbilical catheters have been measured and compared to the documented lengths in the procedure notes for verification of this method. The supposed error by the unclear entry point of the umbilical measurements were avoided by subtracting the distances of the endpoint of the umbilical vessel up the junction of the iliac artery (artery) or the diaphragm (vein), of the documented catheter lengths.

Results

The error for the verification measurements were in the single digits and can be explained by the unclear entry of the umbilical vessels. The wanted cathether lengths ranged from 4.1 cm to 9.7 cm (artery) and 4.5 cm to 12.2 cm (vein) for infants with a birth weight of 380 g to 4.4 kg. The formulas for the catheter lengths in mm for the application of the artificial placenta where: lartery = 0.0101 * m + 41.821 and lvein= 0.0123 * m + 50.468, where m is the birth weight in gram. The coefficients of determination for these formulas are R2artery= 0.45 and R2vein= 0.79.

Conclusions

This represents the first detailed description of the length of umbilical vessels. The data will be used to develop novel large bore access umbilical catheters. Furthermore, the 3D data will be used to create models of umbilical vessels and training phantoms for fluid-dynamic simulations and training, respectively. With this method, we have developed a way to provide a prediction for the vessel-characteristics out of the provided radiographs.

References

1. n/a

Functional Alterations of the Inward Rectifier Potassium Channel Kir2.1 in an Oxidative Stress-Related Model of Aging Neuroglia

Alessia Remigante[1,2], Sara Spinelli[1,2], Angela Marino[1], Michael Pusch[3], Rossana Morabito[1], Silvia Dossena[2]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy.; [2]Institute of Pharmacology and Toxicology, Paracelsus Medical University, Salzburg, Austria.; [3]Biophysics Institute, National Research Council, Genoa, Italy.; Contact: aremigante@unime.it

Objective

Epilepsy is a common brain disease and its prevalence increases with age. Strikingly, about 50% of all epilepsy cases diagnosed in elderly patients (>65 years) are idiopathic. In this context, aging-related oxidative stress (OS) could be regarded to as a possible mechanism involved in epileptogenesis. One of the key functions of glial cells is the spatial buffering of extracellular K^+ ions that are released by excited neurons and transported through glial inwardly rectifying potassium (Kir) channels from extracellular regions of high K^+ to those of low K^+ to inhibit epileptogenesis (2). Among experimental OS-related aging models, long-term exposure to D-Galactose (D-Gal) is considered the most similar to natural aging (3). In this study, we explored the possible beneficial effect of melatonin (Mel), a free radical scavenger, in a D-Gal-induced aging model in glioblastoma U87-MG cells, which express an endogenous inwardly rectifying K^+ current.

Methods

RT-qPCR was utilized to identify the isoform(s) of Kir channels expressed in U87-MG cells. To confirm the molecular identity of the Kir channel underlying the endogenous K^+ currents, the pharmacological inhibitors ML-133 and VU013 were used with the patch clamp technique. Cell viability and parameters of OS, namely the production of ROS, thiobarbituric acid reactive substances, as well as the membrane total sulfhydryl (-SH) groups have been assessed in U87-MG cells exposed to 100mM D-Gal for 24h with or without pre-exposure to 100µM Mel for 24h. The activity of the endogenous antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) was also assayed. The membrane K^+ conductance was measured by the patch-clamp technique in U87-MG cells or, alternatively, in transiently transfected NIH/3T3 cells.

Results

Screening of all 15 Kir isoforms revealed that the predominant transcript in U87-MG cells corresponds to Kir2.1, with minor contribution of Kir4.1. D-Gal exposure did not have an obvious cytotoxicity but resulted in an increased production of ROS and levels of lipoperoxidation, decreased amount of membrane -SH groups, as well as stimulation of the activity of CAT and SOD, all of which are markers of OS. Interestingly, D-Gal treatment was associated with a decrease of the inwardly rectifying K+ currents sensitive to ML-133. Mel pre-treatment prevented D-Gal-induced OS damage, as well as the decrease in the endogenous Kir2.1 current. This latter finding was also confirmed following ectopic expression of Kir2.1 in NIH/3T3 cells. However, no significant alterations in Kir2.1 mRNA and protein expression in D-Gal-treated U87-MG cells were detected.

Conclusions

Our findings show i) a novel Kir2.1 channel modulation that likely occurs in OS conditions; ii) a crucial role of Mel in alleviating OS-induced damage, including the suppression of Kir2.1 ion current. We suggest that the inhibition of Kir2.1 channel in glia cells could alter extracellular K^+ buffering and contribute to neuronal hyperexcitability associated with oxidative stress. Thus, we propose Mel as an excellent candidate to counteract oxidative alterations in epileptogenesis during aging.

References

1. PMID: 30253417; 2.PMID: PMC3508737; 3.PMID: 1538262.

Surgical outcome of supratentorial meningioma patients aged ≥80 years – retrospective international multicenter study

Arwin Rezai[1], Ilari Rautalin[2,3], Christoph J. Griessenauer[1], Mika Niemelä[3], Miikka Korja[3], Christoph Schwartz[1,3]

[1]Department of Neurosurgery, Paracelsus Medical University, Salzburg, Austria; [2]The National Institute for Stroke and Applied Neurosciences, Auckland University of Technology, Auckland, New Zealand; [3]Department of Neurosurgery, Helsinki University Hospital, University of Helsinki, Finland; Contact: c.schwartz@salk.at

Objective
We previously reported that although patients aged ≥80 years requiring neurosurgical treatment of supratentorial meningiomas suffer from a significant risk of postoperative morbidity and increased short-term mortality, functional outcome after surgery improves in most patients within the first year after surgery. Furthermore, we proposed a decision support tool, based on preoperative neurofunctional status and peritumoral edema (PTBE) volume, to identify patients most likely to benefit from surgical resection. This study aims to evaluate our data in a multicenter setting.

Methods
Clinical data were extracted from electronic medical records and neurofunctional status was assessed by the Karnofsky Performance Scale (KPS). Patients were categorized in poor (KPS ≤40), intermediate (KPS 50-70), and good (KPS ≥80) preoperative subgroups. Volumetric analyses of tumor and PTBE were performed; respective volumes were scored as small (<10 cm3), medium (10-50 cm3), and large (>50 cm3). Descriptive statistics and regression analyses were performed.

Results
The study population consisted of 262 patients (57.3% females). Median age at surgery was 83.0 years (range 80.0 to 96.0). Median preoperative KPS was 70 (range 20 to 100); 117 (44.7%) patients were allotted to the good, 113 (43.1%) patients to the intermediate, and 32 (12.2%) patients to the poor subgroup. Median tumor and PTBE volumes were 30.2 cm3 (range 0.5 to 215.0) and 27.3 cm3 (range 0.0 to 408.9), respectively; large PTBE volume correlated with poor preoperative KPS status (p=0.008). The 90-day and 1-year mortality rates were 9.0% and 13.2%. Within the first postoperative year, 101 (38.5%) patients improved, 87 (33.2%) were unchanged, and 74 (28.2%) were functionally worse (including deaths). Each year-increase of age associated with 44% (23–70%) increased risk of 90-day and 1-year mortality. In total, 111 (42.4%) patients suffered from surgery-associated complications. A maximum tumor diameter ≥5cm (odds ratio 1.87 (1.12–3.13)) and large tumor volume (odds ratio 2.35 (1.01–5.50)) associated with an increased risk of surgery-associated complications. Among patients with poor preoperative status and large PTBE, most (58.3%) benefited from surgery.

Conclusions
Patients with poor preoperative status and large PTBE most often showed postoperative improvements. Despite the overall high risk of surgery-associated complications, tumor resection should be considered for symptomatic very old meningioma patients. Our decision support tool may be of help in identifying cases that most likely benefit from surgery.

References
1. Rautalin I, Niemelä M, Korja M. Is surgery justified for 80-year-old or older intracranial meningioma patients? A systematic review. Neurosurg Rev. 2021;44(2):1061-1069.
2. Rautalin I, Schwartz C, Niemelä M, et al. Mortality of surgically treated 80-year-old or older intracranial meningioma patients in comparison to matched general population. Sci Rep. 2021 Jun 1;11(1):11454. doi: 10.1038/s41598-021-90842-y.
3. Schwartz C, Rautalin I, Niemelä M, et al. Symptomatic peritumoral edema is associated with surgical outcome: a consecutive series of 72 supratentorial meningioma patients ≥ 80 years of age. J Neurooncol. 2020;148(1):109-116.

A machine learning model for the accurate prediction of 1-year mortality in transfemoral TAVI patients: A retrospective observational cohort study

Magnus Rottmann[1], Wolfgang Hitzl[2], Thomas Bertsch[3], Matthias Pauschinger[4], Theodor Fischlein[1], Francesco Pollari[1]

[1]Cardiac surgery, Cardiovascular Center, Paracelsus Medical University-Klinikum Nuremberg, Nuremberg, Germany;; [2]Research and Innovation Management (RIM), Team Biostatistics and Publication of Clinical Trial Studies, Paracelsus Medical University, Salzburg, Austria; [3]Institute of Clinical Chemistry, Laboratory Medicine and Transfusion Medicine, Paracelsus Medical University-Klinikum Nuremberg, Nuremberg, Germany; [4]Cardiology, Cardiovascular Center, Paracelsus Medical University-Klinikum Nuremberg, Nuremberg, Germany; Contact: magnus.rottmann@stud.pmu.ac.at

Objective
Since the introduction of the transcatheter aortic valve implantation (TAVI) [1], this procedure was developed and improved further. Consequently the case numbers rose steadily. But with increasing numbers high costs and resource consumption of TAVI are becoming a problem. One way to reduce cost is to release patients earlier if patient safety is not compromised. The aim of this study is to prove, that a machine learning model can accurately predict 1-year-mortality in TAVI patients and thus help physicians decide which patients can be released earlier to save money and resources.

Methods
Routinely acquired data (134 variables) were used from 629 patients, who underwent transfemoral TAVI from 2012 up to 2018. Support vector machines, neuronal networks, random forests, nearest neighbour and Bayes models were used with new, previously unseen patients to predict 1-year mortality in TAVI patients. A genetic variable selection algorithm identified a set of predictor variables with high predictive power.

Results
Univariate analyses revealed 19 variables (clinical, laboratory, echocardiographic, computed tomographic and ECG) significantly influencing 1-year survival. Before applying the reject option, the model performances in terms of negative predictive value (NPV) and positive predictive value (PPV) were similar between all models. After applying the reject option, the random forest model achieved, after excluding 145 of 447 (32%) patients, a NPV of 98% and a PPV of 89%.

Conclusions
Our model can predict the 1-year survival with very high negative and sufficiently high positive predictive value and very high accuracy . The “reject option” allows high performance and a harmonic integration of the machine-learning in the clinical decision process. This algorithm can be useful for selecting patients who are suitable for early discharge following TAVI.

References
1. Cribier A. Development of transcatheter aortic valve implantation (TAVI): a 20-year odyssey. Arch Cardiovasc Dis. 2012 Mar;105(3):146-52.

Global, regional, and national trends of Spinal Injuries 1990-2019: findings from the Global Burden of Diseases (GBD) study 2019

Mahdi Safdarian, Eugen Trinka, Aljoscha Thomschewski

[1]Department of Neurology, Christian Doppler Klinik, Paracelsus Medical University; Contact: m.safdarian@salk.at

Objective
We aimed to measure the global, regional, and national incidence, prevalence, and years lived with disability (YLDs) of spinal injuries from 1990 to 2019 using data from the Global Burden of Diseases, Injuries, and Risk Factors Study.

Methods
Data on incidence, prevalence, and YLDs of spinal injuries were derived systematically from the GBD 2019 study. Using the framework of GBD 2019, we provide numbers and age-standardized rate changes with 95% uncertainty intervals (UIs) for incidence, prevalence, and YLDs of spinal injuries at neck level and below neck level globally and for 21 GBD regions and 204 countries and territories, among all age groups and both sexes from 1990 to 2019. We tried to detect and report meaningful trends based on location, age and gender.

Results
Globally, there were 20.6 million (95% UI: 18.9–23.6) individuals with spinal injuries in 2019. The incidence number of spinal injuries was 909,000 cases (95% UI: 706,952–1,156,409) with an estimated 6.2 million (95% UI: 4.4–8.1) YLDs. There was a significant 81.5% [95% UI: 74.2 to 87.1], 52.7% [95% UI: 30.3 to 69.8] and 65.4% [95% UI: 56.3 to 76.0] increase in the global prevalence, incidence and YLDs numbers of spinal injuries respectively from 1990 to 2019. However, global age-standardised incidence, prevalence and YLDs rates per 100,000 population for spinal injuries, showed slighter changes of 5.8% (95% UI: 2.6–9.5), -6.1% (-17.2–1.5) and -1.5 % (-5.5–3.2) from 1990 to 2019, respectively. There were great geographical variations in the age-standardised rates of spinal Injuries in 2019, in addition to changes from 1990. The incidence increases with age and remains higher than 50,000 cases from 20 to 84 years with two peaks at 30-34 and 50-54 years. The prevalence and YLDs charts; however, show similar patterns, as they both have one peak around 45-59, and 50-54 years, respectively. Globally, the numbers of incidence, prevalence and YLDs have been always more in males from 1990 to 2019 and this difference remains almost stagnant for all the three indexes during this period with a slight increase for both sexes during this period. “Spinal cord lesions at neck level” was more common in comparison to “spinal cord lesions below neck level” in all incidence, prevalence and specially YLDs values.

Conclusions
Epidemiology of spinal injuries tend to shift toward older ages and the number of affected population is increasing worldwide. While age-standardised rates of incidence, prevalence, and YLDs for spinal injuries changed slightly, absolute counts increased substantially from 1990. There is also a wide geographical heterogeneity in demographic, spatial and temporal patterns of spinal injuries, both at national and regional level, which should be considered by the local injury-prevention decision makers to reduce the overall burden of spinal injuries.

References
1. Hartkopp A, Brønnum-Hansen H, Seidenschnur AM, Biering-Sørensen F. Survival and cause of death after traumatic spinal cord injury. A long-term epidemiological survey from Denmark. Spinal cord. 1997;35(2):76-85.
2. Thietje R, Pouw MH, Schulz AP, Kienast B, Hirschfeld S. Mortality in patients with traumatic spinal cord injury: descriptive analysis of 62 deceased subjects. The journal of spinal cord medicine. 2011;34(5):482-7.
3. van den Berg ME, Castellote JM, de Pedro-Cuesta J, Mahillo-Fernandez I. Survival after spinal cord injury: a systematic review. Journal of neurotrauma. 2010;27(8):1517-28.

WHAT IS AN EARLY REVISION? UNDERSTANDING THE CONCEPT OF EARLY REVISION IN TOTAL JOINT ARTHROPLASTY SURGERY. A SYSTEMATIC LITERATURE REVIEW.

Marco Santarelli[1], Michael Engl[1], Kornelia Pomella[1], Simon Demetz[1], Marco Necchi[1], Wolfgang Wirth[2], Mattia Saccoccio[3]

[1]Department of Orthopaedics, Sabes Hospital, South Tyrol; [2]Department of Imaging & Functional Musculoskeletal Research, Institute of Anatomy & Cell Biology, Paracelsus Medical University Salzburg & Nuremberg, Strubergasse 21, 5020, Salzburg, Austria.; [3]Department of Physics, Technical University of Denmark, 2800 Kongens Lyngby, Denmark.; Contact: marco.santarelli@sabes.it

Objective
Revision surgery in Total Joint Arthroplasty is widely recognized as the main performance index. Whereas long-term revision assesses best performing strategies, the concept of “early revision” is not defined clearly and remains a “foggy area”. The latter event mostly occurs within 5 years after index surgery. However, different endpoints are used in the literature ranging from 3 to 6 months until 2 or even 3 years.
Aim of this work is to review systematically the current literature specifically related to “early” revisions, in order to better understand the terminology of “early revision” and allow a rationale for further discussion.

Methods
A systematic literature review of the literature published within the last 10 years with specific keywords retrieved more than 250 articles. Applying the exclusion and inclusion criteria, 105 publications were analysed.

Results
We found a marked heterogeneity in timeframe-choice for early revision among the analysed articles ranging from within 3 months to 5 years. Once the different time frames are set, a comparison among published articles is possible, but data are few. We found 3-months revision rates between 0.5% - 1.5% for THA, and to be around 0.5% for TKA for all causes. 5-year revision rates are reported to be between 2% - 4% for THA and 3% - 5% for TKA for all causes. However, it seems that early revision rates are linked to re-operation rates. A rise in re-operations showed a decrease in revisions rates within the first 2 years in the Swedish Arthroplasty register. Furthermore, we found that the heterogeneity for the applied time definitions in early revisions do not allow a direct comparison as the different causes for revisions usually occur at different time points specific for that cause.

Conclusions
We conclude that as long as we do not integrate the specific cause for early revision, a direct comparison between the published data is not possible unless we want to compare apple with pears. Therefore, we suggest a common set of defined parameters for each cause of early revision and the inclusion of reoperation rates to compare early revision rates.

References
1. Cnudde, P. et al. Trends in hip replacements between 1999 and 2012 in Sweden. Journal of Orthopaedic Research 36, 432–442 (2018).
2. Evans, J. T. et al. How long does a hip replacement last? A systematic review and meta-analysis of case series and national registry reports with more than 15 years of follow-up. Lancet 393, 647–654 (2019).
3. Singh, J., Politis, A., Loucks, L., Hedden, D. R. & Bohm, E. R. Trends in revision hip and knee arthroplasty observations after implementation of a regional joint replacement registry. Canadian Journal of Surgery 59, 304–310 (2016).

Comparison of endoscopic lumbar laminoforaminotomy versus conventional microsurgical technique for the treatment of lumbar spinal stenosis

Babak Saravi[1,2,3], Gernot Lang[1,2], Sara Ülkümen[1,2], Sebastien Couillard-Despres[3], Frank Hassel[2]

[1]Department of Orthopedics and Trauma Surgery, Medical Centre—Albert-Ludwigs-University of Freiburg, Faculty of Medicine, Albert-Ludwigs-University of Freiburg, Freiburg, Germany; [2]Department of Spine Surgery, Loretto Hospital, Freiburg, Germany; [3]Institute of Experimental Neuroregeneration, Paracelsus Medical University, Salzburg, Austria; Contact: babak.saravi@hotmail.de

Objective
Endoscopic spine surgery is a promising, minimally invasive technique for the treatment of foraminal and spinal stenosis (1). However, literature on the efficiency of endoscopic lumbar laminoforaminotomy (ELF) versus conventional microsurgical technique (CMT) in patients with both, lumbar foraminal and spinal stenosis is scarce. The present study sought to conduct a case-controlled comparison regarding treatment success with consideration of clinical and radiologic parameters.

Methods
We included 28 consecutive patients (n=14 ELF, n=14 CMT) presenting with both foraminal and lumbar central spinal stenosis between 2018-2020. Surgery-related (surgery time, complications, hospital length of stay (LOS), ASA score, C-reactive protein (CRP), white blood cell count (WBCC), patient-related outcome measures (PROMs) (ODI, NRS (leg-, back pain), eQ5D, COMI), and radiological (preoperative dural sack cross-sectional area (DSCA), Shizas score (SC), pre- and postoperative foraminal height (FH), and foraminal area (FA)) parameters were examined and compared between the two groups. PROMs were assessed preoperatively, three weeks postoperatively and 1-year postoperatively.

Results
Complication (most often re-stenosis due to hematoma and/or residual sensorimotor deficits) rates were higher in the endoscopic (28.6%) than microsurgical (7.1%) treatment group, although not significantly ($p=0.139$). Age, ASA score, preoperative DSCA, FH, FA, and Shizas score as potential confounding variables did not significantly differ between the study groups. Mean surgery time was 67.6±4.1 min for ELF and 50.9±6.0 min for CMT ($p<0.05$). Mean LOS was 9.1± 2.7 days for ELF and 14.4±2.2 days for CMT ($p<0.01$). FH and FCSA did not reveal significant differences compared to preoperative measured values for both groups. WBCC ($p<0.0001$) and CRP ($p<0.05$) were significantly increased postoperatively for CMT but not for ELF. Three weeks and 1 year postoperative evaluated ODI, COMI, eQ5D, NRS leg, or NRS back values revealed significant improvements for the ELF group. However, we did not observe significant differences between ELF and CMT for the PROMs for any of the examined time points.

Conclusions
Endoscopic treatment of lumbar spinal stenosis was associated with longer operation time and higher complication rates in our single-center study experience. This was probably because of the surgeons' lack of experience with this method and the resulting different learning curve compared with the conventional technique. However, ELF resulted in lower postoperative WBCC and CRP and shorter THR, emphasizing its minimal invasiveness. Regarding the PROMs, it was similar successful as the conventional microsurgical approach. The endoscopic technique could be a new gold standard in the treatment of lumbar spinal stenosis if surgeons are sufficiently trained.

References
1. Park J, Ham DW, Kwon BT, Park SM, Kim HJ, Yeom JS. Minimally Invasive Spine Surgery: Techniques, Technologies, and Indications. Asian Spine J. 2020 Oct;14(5):694-701. doi: 10.31616/asj.2020.0384. Epub 2020 Oct 14. PMID: 33108835; PMCID: PMC7595822.

Comparison of Advanced Practice Nursing in North American and German-Speaking European Countries

Firuzan Sari Kundt[1], Margitta Beil-Hildebrand[1]

[1]Institute of Nursing Science and Practice, Paracelsus Medical University, Salzburg, Austria; Contact: firuzan.sari@pmu.ac.at

Objective
This project compares the professionalization and educational standards of Advanced Practice Nursing (APN) in the USA, Canada, Germany, Austria and Switzerland with specific attention to geographical, educational and professional factors influencing the evolution of the APN role.

Methods
We performed a review of the literature, scientific articles, governmental regulatory texts and legislative codes from each country. Patterns related to the geographical, educational and professional context of nursing with comparative insights on the evolution of the discipline in each country, were identified.

Results
USA: APN education, licensure and scope of practice laws vary a great deal between states. There is continued effort that all states should allow APNs to practice autonomously within their specified role in addition to requiring a doctor of nursing practice (DNP) degree for all APNs.
Canada: Nursing is a self-regulated discipline in Canada, with provincial and territorial government ensuring self-regulating authority. As of 2021, all APNs in Canada were required to be educated at the graduate level and pass a certification exam administered by provinces and territories.
Germany: Development of the APN role in Germany has faced strong opposition from the medical community. The first APN master's degree in Germany was launched in 2007 and the first APN position was developed in 2009. As of 2021, APNs in Germany are employed in clinical care, as translators of evidence, supervisors and nursing educators as well as researchers.
Austria: Although the first doctor of philosophy (PhD) program in nursing science was offered in 2000 at the Medical University of Graz, neither the bachelor's nor the doctoral degree qualified graduating nurses to go into APN. The first postgraduate studies began in 2012 as nonconsecutive APN programs.
Switzerland: Master's and doctoral level degrees are offered and PhD programs exist since 2005, but curricula are not yet standardized across programs. APNs in most of Switzerland do not practice autonomously. They function primarily in roles such as clinical leadership, practice improvement and quality care. Since 2018, master's level APNs in Switzerland participate in clinical care, research and health promotion.

Conclusions
Despite the wide variance between the status of autonomous APN in North American and German-speaking European countries and progression at differing paces, a similar pattern of professionalization is present in each country. The U.S. has progressed the furthest towards establishing APN independent of medicine, followed by Canada and Switzerland, and lastly Germany and Austria being in similar preliminary stages of progression. Countries with a longer history of academization and standardization of educational requirements for APN have made greater progress towards APN autonomy.

References
1. Beil-Hildebrand, M.B. & Smith, H.B. (2022). Comparative Analysis of Advanced Practice Nursing: contextual and historical Influences in North American and German-speaking European countries. Policy, Politics and Nursing Practice, Vol 23, No 3, pp 162-174. DOI: 10.1177/15271544221105032

IL-10 family cytokines in the interrelation of osteoarthritis and type 2 diabetes mellitus (Bedeutung von Zytokinen der IL-10 Familie in der Wechselbeziehung zwischen Osteoarthritis und Diabetes mellitus Typ 2)

Teresa Schotte[1], Julius Wehrmann[1], Clemens Gögele[1], Christian Werner[1], Michael Wagner[2], Hermann Josef Bail[2], Gundula Schulze-Tanzil[1]

[1]Institute of Anatomy and Cell Biology, Paracelsus Medical University, Nuremberg, Germany; [2]Department of Orthopedics and Trauma Surgery, Paracelsus Medical University, Nuremberg, Germany; Contact: Teresa.Schotte@stud.pmu.ac.at

Objective
Osteoarthritis (OA) is the most common joint disease and a widespread cause of pain and loss of function[1,2]. The pathophysiological relationship between OA and type 2 diabetes mellitus (DMT2) raises a number of research questions, as both comprise similar processes. The secretion of proinflammatory cytokines results in low-grade systemic inflammation in both diseases[3,4]. The complement activation detected in OA pathogenesis, especially the anaphylatoxin C5a, could also play a role in DMT2. Since it is a research goal to be able to use both rat chondrocytes and chondrocytes of human donors for the research of both diseases, chondrocytes of both species were first examined in their response to stimulation with proinflammatory tumor necrosis factor α (TNFα, rat, human) in order to draw conclusions for the use of rat chondrocytes in a diabetic OA model. So far, the role of cytokines of the interleukin (IL)-10 family in the interrelation of both diseases has been discussed. Therefore attempts have been made to regulate anti-inflammatory IL-10 and IL-24 when chondrocytes are exposed to hyper- and normoglycemic culture medium and in the presence of the proinflammatory factors TNFα and C5a.

Methods
Cultured human and rat chondrocytes were treated with hyper- and normoglycemic stimulation medium (+/-TNFα and +/-C5a). Vitality analysis was performed with propidium iodide and fluoresceindiacetate. In addition, the influence of glucose supply on metabolic activity, synthesis of matrix proteins, cytokines and complement associated proteins as well as gene expression was investigated.

Results
The applicability of rat chondrocytes in an *in vitro* osteoarthritis model was comparable to that of chondrocytes derived from human donor cartilage. After stimulation with TNFα, chondrocytes of both species showed induced gene expression of IL-6 and significantly suppressed collagen type 1 and type 2 expression. Furthermore, the induction of IL-10 (gene and protein expression) and IL-24 (protein expression tested only) was demonstrated in human chondrocytes under stimulation with TNFα and C5a (in normoglycemia).

Conclusions
The finding that rat chondrocytes respond similarly to human and rat TNFα helps to evaluate published results obtained with human TNFα in rat chondrocytes. The regulation of IL-10 and IL-24 by TNFα and the complement cleavage product C5a is still a trend, which needs to be statistically validated in the future with the inclusion of further cell isolates.

Acknowledgements
The authors would like to thank *Stiftung Endoprothetik* and *Schöllerstiftung* for providing financial resources for this research work.

References
1. Schulze-Tanzil, 2019. Cells. Vol. 8, 990. PMC6769780
2. Centers for Disease Control and Prevention, 2010. Morbidity and Mortality Weekly Report. Vol. 59, 1261-5
3. Schwarz et al, 2018. Inflammation Research. Vol. 67, 285-300. DOI: 10.1007/s00011-017-1121-8
4. Courties, Sellam, 2016. Diabetes Research and Clinical Practice. Vol. 122, 198-206. DOI: 10.1016/j.diabres.2016.10.021

Pelvic Organ Prolapse Treatment by Unilateral Pectineal Suspension

Michael Schreibmayer[1,2], Dimitrios Bolovis[1], Cosima Brucker[1], Wolfgang Hitzl[3]

[1]Department of Gynecology and Obstetrics, Nuremberg General Hospital, Paracelsus Medical University, Nuremberg, Germany; [2]Department of Gynecology and Obstetrics, Krankenhaus der Barmherzigen Brüder, Academic Teaching Hospital, St. Veit/Glan, Austria; [3]Department of Ophthalmology and Optometry, Paracelsus Medical University, Salzburg, Austria; Contact: michael.schreibmayer@gmail.com

Objective
The Unilateral Pectineal Suspension, is a novel technique for apical and combined prolapse repair fulfilling a broad range of quality criteria for pelvic organ prolapse (POP) repair. UPS provides mesh-free midline anatomical suspension of the uterus using a single unilateral non-absorbable suture connecting the anterior cervix to the lateral part of the iliopectineal ligament. The purpose of this retrospective study was to analyze perioperative complication rate, short-term efficiency, and overall patient acceptance of the new UPS surgical concept.

Methods
In the period of 1st of January 2020, to 1st of January 2022, 47 consecutive patients presenting with isolated or combined apical prolapse were scheduled for surgical treatment with UPS. All patients were examined during a routine follow-up visit 3-6 months after surgery to determine treatment success as defined by a composite endpoint. The composite endpoint was defined as an apical pelvic organ prolapse stage ≤ 1.

Results
Mean patient age was 63.6 years (range 35-84 years). Mean BMI was 24.6 kg/m2 (range 17.8-43.2 kg/m2). The patients stayed in the hospital 4 days on average (range 2-13 days). No drains were used. No conversions were observed. Estimated blood loss as shown by decrease in hemoglobin levels was 0.89 g/dl. Mean operating time for UPS alone was 44.08 min (range 20-74 min). Mean duration of UPS with additional procedures (anterior repair, posterior repair, supracervical hysterectomy, BURCH colposuspension) was 82.97 min (range 37-235 min). The longest operation time observed included extensive adhesiolysis and BURCH colposuspension. On follow-up examination after 3 to 6 months the composite endpoint was reached in 44 of 47 patients (93.6%). Patient satisfaction resembled the results of the composite endpoint.

Conclusions
The new surgery method Unilateral Pectineal Suspension combines a minimal invasive, unilateral, mesh free suspension technique for isolated or combined apical POP correction. UPS can be combined with additional procedures for the correction of POP and / or stress urinary incontinence during the same or a subsequent procedure. Unilateral Pectineal Suspension demonstrates improvement in pelvic floor function and support. It is a save technique with excellent results in short term follow up examination.

References
1. Banerjee C, Noé KG. Laparoscopic pectopexy: a new technique of prolapse surgery for obese patients. Arch Gynecol Obstet. 2011 Sep;284(3):631-5. doi: 10.1007/s00404-010-1687-7. Epub 2010 Oct 13. PMID: 20941503.
2. Haylen BT, Maher CF, Barber MD, Camargo S, Dandolu V, Digesu A, Goldman HB, Huser M, Milani AL, Moran PA, Schaer GN, Withagen MI. An International Urogynecological Association (IUGA) / International Continence Society (ICS) joint report on the terminology for female pelvic organ prolapse (POP). Int Urogynecol J. 2016 Feb;27(2):165-94. doi: 10.1007/s00192-015-2932-1. PMID: 26755051.
3. Bolovis DI, Brucker CVM. Unilateral pectineal suspension - A new surgical approach for apical correction of pelvic organ prolapse. Facts Views Vis Obgyn. 2022 Jun;14(2):177-181. doi: 10.52054/FVVO.14.2.015. PMID: 35781115.

A Neurofilament-L Reporter Cell Line for the Quantification of Early Neuronal Differentiation: A Bioassay for Neurotrophic Activities

Lisa-Franziska Seidl[1,2], Stefan Winter[2], Ludwig Aigner[1], Julia Schartner[2]

[1]Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg, Austria; [2]EVER Neuro Pharma GmbH, Oberburgau 3, 4866 Unterach am Attersee, Austria; Contact: lisa.seidl@stud.pmu.ac.at

Objective
Neurotrophic activity constitutes a crucial factor in the recovery from neurological injuries and is impaired in neurodegenerative disorders. Preclinical studies of neurotrophic factors to improve outcome of neurodegenerative diseases have yielded promising results. However, due to the complexity of these therapies, the clinical translation of this approach was so far not successful and more feasible treatments with neurotrophic activity may be promising alternatives. Therefore, highly sensitive and robust assays for compound screening are required.

Methods
One aspect of neurotrophic activity, neuronal differentiation, can be evaluated by the quantification of neurite outgrowth in a rat pheochromocytoma cell line (PC12 cells), an approach that is time-consuming and of high variability. It was already shown that the expression levels of Neurofilament-L (NF-L), a marker of early neuronal differentiation, correlate with neurite outgrowth in PC12 cells. Thus, we generated and characterized an EGFP-NF-L reporter PC12 cell line for the development of a cell-based assay that allows straightforward evaluation of neurotrophic activity based on NF-L expression.

Results
Using Cerebrolysin® as a model for a pharmacological compound that stimulates neurotrophic activity in the central nervous system, the EGFP-NF-L reporter PC12 cell-based assay was proven to be a robust, specific, and reproducible method.

Conclusions
This work describes the development of an EGFP-NF-L reporter PC12 cell-based assay as a robust and reproducible tool for compound screening for neurotrophic activity with relatively high throughput. Moreover, it could serve as a model system for studying Neurofilament-L signaling.

References
1. Schimmelpfeng J, Weibezahn KF, Dertinger H. Quantification of NGF-dependent neuronal differentiation of PC-12 cells by means of neurofilament-L mRNA expression and neuronal outgrowth. J Neurosci Methods. 2004;139(2):299-306.

A histological analysis and detection of complement regulatory proteins CD55 and CD59 in SARS-CoV-2 infected lungs

Sandeep Silawal[1]

[1]Institute of Anatomy and Cell Biology, Paracelsus Medical University, Nuremberg and Salzburg, General Hospital Nuremberg, Prof. Ernst Nathan Str. 1, 90419 Nuremberg, Germany; [2]Institute for Pathology, Paracelsus Medical University, Nuremberg, General Hospital, Prof. Ernst Nathan Str. 1, 90419 Nuremberg, Germany; [3]Department of Emergency and Intensive Care Medicine, Klinikum Ernst von Bergmann, Charlottenstraße 72, 14467 Potsdam, Germany; Contact: sandeep.silawal@pmu.ac.at

Objective
A complement imbalance in the alveolar and/or vascular tissue can play a deteriorating role in COVID-19, leading to an acute respiratory distress syndrome (ARDS). CD55 and CD59 are membrane attached complement regulatory proteins functioning at different levels of the complement cascade

Methods
Lung specimens from COVID-19 and ARDS positive (+COVID/+ARDS) patients were compared with COVID-19 and ARDS negative (-COVID/-ARDS) as well as -COVID/+ARDS patients. Histochemical staining, immunofluorescence and immunohistological staining of CD55 and CD59 proteins respectively and scanning electron microscopy imaging were performed.

Results
-COVID/-ARDS specimen showed higher expression, homogeneous distribution of glycosaminoglycan and compactly arranged, collagen fibers on the alveolar walls in comparison to ARDS affected lungs. CD55 expression in lung tissues coincided with the collagen distribution pattern, suggesting CD55 deposition on the alveolar collagen. -COVID/-ARDS lung tissues showed homogenously distributed CD55 expression on the alveolar walls in comparison to the disrupted +COVID/+ARDS lung tissue. CD55 protein expression could not be detected in the smooth muscles of lung blood vessels. A low CD59 expression found in the endothelial linings of lung tissues can be related to the severity of the lung damage.

Conclusions
The complement regulatory proteins, CD55 and CD59 expression could play overall a protective role in the SARS-CoV-2 infected lungs. A larger patient collective has to be investigated in the future.

Acknowledgements
We would like to thank Dr. Ernst & Anita foundation for the financial support and Ms. Anke Hochholzer for the technical support.

References
1. Huang, C., et al., Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet, 2020. 395(10223): p. 497-506
2. Janeway Jr, C.A., et al., Immunobiology: The Immune System in Health and Disease. 5th edition. 2001, New York: Garland Science

Innovationsfond-Projekt UPlusE "U-examination for children PLUS parents at the pediatrician to promote child development"

Susanne Simen[1], Sarah Kittel-Schneider[2], Katharina Kuscher[1], Wolfgang[3], Susanne Rauber[4], Ulf Dammer[5], Ronny Jung[6], Lea Schröder[7], Torsten Schröder[7], Neslisah Yilmaz-Terzioglu[8], Neeltje van den Berg[8], Christoph Fusch[9]

[1]Department of Psychiatry and Psychotherapy, Paracelsus Medical University, Nuremberg, Germany; Department of Psychiatry, Psychotherapy and Psychosomatic; University Würzburg; [3]Department of Obstetrics, , Paracelsus Medical University, Nuremberg, Germany; [4]Department of Obstetrics and Antenatal Medicine, Clinic Hallerwiese, Nuremberg, Germany; [5]Department of Gynecology and Obstetrics, St. Theresien Hospital, Nuremberg, Germany; [6]Outpatient pediatrician, Roth, Germany; [7]Praenatal, Prenatal Medicine, Gynaecology & Genetic, MVZ GbR, Nuremberg, Germany; [8]Department of Epidemiology of Health Care and Community Health, Institute for Community Medicine, University Greifswald, Greifswald, Germany; [9]Department of Neonatology, Children and adolscents, Paracelsus Medical University, Nuremberg, Germany; Contact: susanne.simen@klinikum-nuernberg.de

Objective
Mental illnesses in parents during peripartum can have a negative impact on the children's development. Therefore the UPlusE project, funded by the G-BA Innovation Fund with 4.6 million euros, will provide a screening for perinatal depression of pregnant women by gynaecologists and of parents by paediatricians - and mediation of mental health treatment. We aim to examine whether utilization of mental health treatment increases among identified parents compared with a control group.

Methods
In UPlusE, the well established practice apps of paediatric and gynaecological practices are used for Screening bei short validated questionnaires about depression (EPDS). Affected mothers are referred to mental health treatment. The project will include 10,000 parents throughout Germany who are insured at BKK. Consortium leadership is provided by the Department of Psychiatry and Psychotherapy and the Department of Newborn, Children and Adolescents, PMU Nuremberg.

Results
The UPlusE project and the results of the regional pilot project in Nuremberg are presented. Over 5,000 women were screened using the EPDS-Plus within 21 months. The prevalence of depression in the peripartum period was 10%. Experiences of violence were reported by 11%, and 20% had experienced the births of their child as very stressful to traumatic. The acceptance by physicians and patients was very high.

Conclusions
We expect the UPlusE project to increase the use of mental health treatment and support, and thereby to improve the mental health of young families and to reduce the transgenerational transmission of mental illness in stressed families and individual suffering in the longer term.

Acknowledgements
We thank all participating maternity hospitals and medical practices that enabled the pilotstudy and set the ground for UPlusE. And we thank G-BA for funding UPlusE.

References
1. Simen et al. (2023). Routine screening for peripartum depression in the gynaecologic and paediatric setting - evaluation of an adapted EPDS version. Zeitschrift für Geburtshilfe und Neonatologie (ZGN) 1

Interatrial block paves the way to postoperative atrial fibrillation after cardiac surgery in patients with preoperative sinus rhythm

Sluet P[1], Leiler S[1,2], Bernik R[3] , Bauer A[4] , Günzler V[1] , Angerer [1] , Fischlein T [1,2] , Kalisnik JM[1,2]

[1]Paracelsus Medical University, Nuremberg, Germany, [2]Department of Cardiac Surgery, Paracelsus Medical University, Nuremberg, Germany, [3]Clinic of Neurology, University Medical Centre Ljubljana, Slovenia, [4]Department of Computer Sciences, Germany University of Würzburg, Germany

Objective
Following cardiac surgery, postoperative atrial fibrillation is a common complication which leads to adverse outcomes, higher mortality and prolonged hospital stays. While yet a little renowned entity, the interatrial block became evident as a potential risk factor for atrial fibrillation in the recent past. This study aims to clarify the role of the interatrial block in the development of postoperative atrial fibrillation to improve patient outcome and to validate its worth as a potential risk factor to be considered in pre-operative risk stratification.

Methods
We examined pre- and postoperative ECG-findings of 1350 patients to identify existing interatrial blocks and to observe the development of postoperative atrial fibrillation in patients undergoing aortic-, mitral- or tricuspid valve replacement or reconstruction, coronary artery bypass grafting or a combination of these procedures. Patients have been followed during clinical stay until discharge. Interatrial blocks have been classified according to definitions of Bayes de Luna et al.

Results
Patients with existing interatrial blocks of any kind develop atrial fibrillation at a higher rate than patients without this condition (OR 2,30; CI 1,75-3,03; p=<0,001). When exhibiting advanced interatrial block, the rates are even higher (typical advanced IAB: OR 2,80; CI 1,34-6,31; p=0,008). Other risk factors include a preexisting left bundle branch block (OR 2,42), hypertension (OR 1,41), female gender (OR 1,37), and high ratings regarding EuroSCORE II (OR 1,12).

Conclusions
The interatrial block and especially its higher grades are a risk factor for the development of postoperative atrial fibrillation. Due to its easy and cheap obtainability within most cases already given diagnostic tools, it can be a valuable addition in pre-operative risk assessment. Further studies are needed to evaluate its predictive value for different types of intervention.

EARLY ALLOGENEIC STEM CELL TRANSPLANTATION AFTER MELPHALAN INDUCED APLASIA LEADS TO LONG-TERM SURVIVAL IN PATIENTS WITH PRIMARY REFRACTORY AML

Claudius Söhn[1], Kerstin Schäfer-Eckart[1], Knut Wendelin[1], Wolfgang Hitzl[2], Stefan Knop[1]

[1]Department of Internal Medicine, Hematology and Oncology, Klinikum Nuremberg, Nuremberg, Germany; [2]Department of Research and Innovation Management, Biostatistics and Publication of Clinical Trial Studies, Paracelsus Medical University, Salzburg, Austria; Contact: claudius.soehn@alumni.pmu.ac.at

Objective
Survival rates of younger patients with newly diagnosed acute myeloid leukemia (AML) have improved, but still up to 30% of patients (pts) fail to achieve complete remission after induction therapy. Allogeneic blood stem cell transplantation is the only option for long-term remission in eligible patients. Salvage chemotherapy before allogeneic hematopoietic stem cell transplantation (HSCT) is considered as a standard of care by most centers. Yet this approach is associated with considerable treatment-related complications and only about 50% of patients will achieve CR before transplantation (1). Here we present our results of an immediate allogeneic transplantation in refractory disease after chemotherapy-induced aplasia.

Methods
All pts from our center with refractory AML after induction chemotherapy who received an allogeneic transplantation in active disease between 2012 and 2021 were retrospectively analyzed. Overall survival was evaluated in regard to time from diagnosis to HSCT using Cox proportional hazard model. The use of one induction cycle vs. two induction cycles after diagnosis was analyzed in regard to overall survival using Kaplan-Meier analysis.

Results
41 pts were included. Their median age was 56 (range 24-75) years. Melphalan (100-140 mg/m) for induction of aplasia was used in 36/41 pts. The conditioning regimen consisted of Treosulfan (30g/m) and Fludarabine (150mg/m) in most pts (68%). GvHD-prophylaxis was CSA and MTX or CSA and MMF. 34 pts (83%) additionally received ATG-prophylaxis. Mean time from diagnosis to HSCT was 3 (range 0.93-11.03) months (mos). Induction chemotherapy was daunorubicin and cytarabine (DA 3+7) in all pts. 17 pts received 1 cycle; 2 induction cycles were given to 24 pts. 22 of those 24 pts were given high-dose AraC as second induction because of refractory disease after first induction. After a median follow-up of 8.9 (range 0.3-122) mos 14 pts (34.1 %) were alive without relapse. Median observation time for those event free at the last follow up (14/41 patients) was 41 mos (range 7-122 mos). This was considerably longer than the median relapse-free survival of 9.2 mos. The last relapse took place 18 mos after transplantation. The main cause of death was early treatment-related mortality (59 %) mainly because of septicemia (44 %). Only 7 pts (17 %) relapsed and 2 pts (7 %) had refractory disease. Cox proportional hazard model showed no significant correlation between time from diagnosis to HSCT and overall survival (p=0.19). There was no significant difference of 1 vs. 2 Induction cycles in regard to OS (p=0.51).

Conclusions
In this retrospective analysis, we showed that an immediate allogeneic transplantation after induction of aplasia is feasible in pts with primary refractory AML. With a two-year survival rate of 37%, this approach offers a realistic chance to patients with an otherwise dismal prognosis. The effectivity is compromised by the high rate of early toxicity mainly because of infectious reasons.

References
1. Othus M, et al. Fate of patients with newly diagnosed acute myeloid leukemia who fail primary induction therapy. Biol Blood Marrow Transplant. 2015;21(3):559-564. doi:10.1016/j.bbmt.2014.10.025. PMID: 25536215

18F-FET-PET MULTIPARAMETRICAL ANALYSIS FOR DIFFERENTIATING DISEASE RELAPSE AND PSEUDOPROGRESSION IN REAL-LIFE HIGH GRADE GLIOMA PATIENTS

Alessandro Spimpolo[1], Davide Drusiani[2], Leda Lorenzon[3], Paolo Cipriano Cecchi[2], Andreas Schwarz[2], Mohsen Farsad[1]

[1]Department of Nuclear Medicine, Hospital of Bolzano-Bozen (SABES-ASDAA), Teaching Hospital of the Paracelsus Medizinischen Privatuniversität (PMU), Bolzano-Bozen, Italy; [2]Department of Neurosurgery, Hospital of Bolzano-Bozen (SABES-ASDAA), Teaching Hospital of the Paracelsus Medizinischen Privatuniversität (PMU), Bolzano-Bozen, Italy; [3]Department of Medical Physics, Hospital of Bolzano-Bozen (SABES-ASDAA), Teaching Hospital of the Paracelsus Medizinischen Privatuniversität (PMU), Bolzano-Bozen, Italy; Contact: alessandro.spimpolo@sabes.it

Objective
In operated High-Grade-Glioma (HGG) patients, 18F-FET-PET represents a useful tool to differentiate pseudoprogression from disease recurrence after adjuvant chemoradiotherapy. The most reliable 18F-FET-PET-derived parameter, however, is still debated. The aim of our study was to retrospectively evaluate the most accurate parameter in our patient population and compare its value with European Guidelines reference.

Methods
From 2017 to 2022, 22 consecutive HGG patients underwent 23 dynamic 18F-FET-PET after surgical resection and adjuvant chemoradiotherapy. The Tumour-to-background ratio (TBR) cut-off value of 1.6 was used to automatically segment tumour volume (MTV) in visually positive studies. A standard VOI (VOIst) was centred on the region of maximum tumour uptake. Thus, dividing the mean uptake of VOIst and MTV by background, TBRmax and TBRmean were calculated, respectively. Time-activity curves (TACs) of VOIst were plotted and their shape classified as increasing (Type1), plateau (Type2) or decreasing (Type3) with Type1 considered as negative and Type2 or Type3 as positive. Histological data and/or MRI 3 months after 18F-FET-PET were used as gold standard to distinguish between relapse and pseudoprogression.

Results
17/23 (74%) scans were classified as relapsing while 6/23 (26%) as pseudoprogressive, based on follow-up data (9/23) and histology (14/23). ROC curve analysis of SUVmax, MTV, TBRmax and TBRmean showed an AUC of 0.84, 0.56, 0.81 and 0.81 and optimal cut-off values, sensitivity and specificity were (2.34, 77%, 83%), (3cm3, 63%, 67%), (1.49, 82 %, 83%), and (1.73, 88%, 67%). TACs-pattern analysis showed 71% sensitivity and 83% specificity. If the guidelines (1) reported cut-off value of 1.9 had been used for TBRmax, sensitivity and specificity would have been 68% and 83%, respectively.

Conclusions
In HGG, the 18F-FET-PET-derived parameter TBRmax proved to be an accurate imaging tool for the differential diagnosis between pseudoprogression and disease relapse but its optimal cut-off should still be determined on a larger population.

References
1. 1. Law I, Albert NL, Arbizu J, Boellaard R, Drzezga A, Galldiks N, et al. Joint EANM/EANO/RANO practice guidelines/SNMMI procedure standards for imaging of gliomas using PET with radiolabelled amino acids and [18 F]FDG: version 1.0. Eur J Nucl Med Mol Imaging. 2019;46(3). PMID: 30519867

Age-related oxidative stress impairs the activity of Kv3.1 channel: protective role of melatonin

Sara Spinelli[1,2], Alessia Remigante[1], Gianluca Mantegna[1], Angela Marino[1], Rossana Morabito[1], Silvia Dossena[2]

[1]Dept Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina, Italy; [2]Institute of Pharmacology and Toxicology, Paracelsus Medical University, Salzburg, Austria; Contact: saspinelli@unime.it

Objective

Kv3.1 (KCNC1) is a Shaw-type delayed rectifier potassium channel. Genetic mutation of Kv3.1 causes progressive myoclonic epilepsy (PME) (1,3) and its expression in neurons of the rat central auditory system decreases with age (2,5,4). Since oxidative stress (OS) is thought to play a key role in epilepsy as well as age-related hearing loss, the purpose of the present study is to assess whether age-related oxidative stress can affect Kv3.1 channel function and evaluate the potential beneficial effect of melatonin (Mel), a neurohormone with strong antioxidant activity.

Methods

Human kidney cells (HEK293 Phoenix) were exposed for 48 h to 100 mM D-Gal, with or without 100 µM Mel, or incubated with 1 mM H_2O_2 for 30 min to induce OS. Kv3.1 activity was evaluated by whole-cell patch-clamp on the endogenous (HEK293 Phoenix) and ectopic (NIH/3T3 fibroblasts) Kv3.1 currents. Cell viability and markers of OS, including thiobarbituric acid reactive substance (TBARS) levels, total content of sulfhydryl (SH) groups, reactive oxygen species (ROS) production, and superoxide dismutase (SOD) and catalase (CAT) activity have been analysed.

Results

Our results show that: i) exposure of cells to D-Gal did not affect cell viability, increased all OS markers, and significantly inhibited Kv3.1 currents; ii) H_2O_2 did not significantly affect Kv3.1 currents, thus indicating that the channel is not a direct target of OS; iii) Mel restored alterations of OS markers induced by exposure to D-Gal and prevented Kv3.1 current reduction.

Conclusions

These data contribute to clarify the molecular mechanisms of age-related hearing loss and epilepsy and further suggest that antioxidant strategies might be useful to counteract these aging-related diseases. Further studies are needed to verify the molecular pathways that impair channel activity.

References

1. Carpenter JC, Männikkö R, Heffner C, Heneine J, Sampedro-Castañeda M, Lignani G, Schorge S. Progressive myoclonus epilepsy KCNC1 variant causes a developmental dendritopathy. Epilepsia. 2021 May;62(5):1256-1267. doi: 10.1111/epi.16867. Epub 2021 Mar 18. PMID: 33735526; PMCID: PMC8436768.
2. Peixoto Pinheiro B, Vona B, Löwenheim H, Rüttiger L, Knipper M, Adel Y. Age-related hearing loss pertaining to potassium ion channels in the cochlea and auditory pathway. Pflugers Arch. 2021 May;473(5):823-840. doi: 10.1007/s00424-020-02496-w. Epub 2020 Dec 17. PMID: 33336302; PMCID: PMC8076138.
3. Munch AS, Saljic A, Boddum K, Grunnet M, Hougaard C, Jespersen T. Pharmacological rescue of mutated Kv3.1 ion-channel linked to progressive myoclonus epilepsies. Eur J Pharmacol. 2018 Aug 15;833:255-262. doi: 10.1016/j.ejphar.2018.06.015. Epub 2018 Jun 9. PMID: 29894724.
4. Peixoto Pinheiro B, Vona B, Löwenheim H, Rüttiger L, Knipper M, Adel Y. Age-related hearing loss pertaining to potassium ion channels in the cochlea and auditory pathway. Pflugers Arch. 2021 May;473(5):823-840. doi: 10.1007/s00424-020-02496-w. Epub 2020 Dec 17. PMID: 33336302; PMCID: PMC8076138.
5. Martin del Campo HN, Measor KR, Razak KA. Parvalbumin immunoreactivity in the auditory cortex of a mouse model of presbycusis. Hear Res. 2012 Dec;294(1-2):31-9. doi: 10.1016/j.heares.2012.08.017. Epub 2012 Sep 23. PMID: 23010334.

Development of a GPU-based simulation framework to investigate electrical excitation waves during cardiac arrhythmia

Daniel Suth[1], Thomas Lilienkamp[1]

[1]Computational Physics Group, Technische Hochschule Nürnberg, Nuremberg, Germany; Contact: daniel.suth@th-nuernberg.de

Objective

In the field of cardiac dynamics, numerical simulations play an important role in understanding fundamental mechanisms and features of the dynamics within the myocardium. Of particular interest are chaotic processes of cardiac dynamics, such as ventricular fibrillation, and the investigation and improvement of methods for their treatment. Our group is focused on developing a numerical simulation framework from scratch to enable unrestricted investigation of a broad range of topics without the limitations imposed by closed source frameworks. By utilizing GPUs, we can achieve high computational performance, enabling us to explore complex topics. Our group is passionate about advancing the state of the art in various areas of research, particularly in the following topics by utilizing numerical simulations: 1) Derive an enhanced understanding of the dynamics during episodes of ventricular fibrillation. 2) Develop concepts for the improvement of current defibrillation techniques and suggestions for completely new strategies

Methods

The propagation of electrical waves in cardiac tissue as an excitable medium can be described by PDEs. We investigated all performance measures for different ionic cell models, namely the Barkley(1), the Fenton Karma(2), the BOCF(3), and the Ten Tusscher(4) model.

Results

All numerical experiments were performed in 2D, 3D, and 3D with realistic heart geometry domains. The usage of GPUs provides significant speedup (approximately by a factor of 2 to 38) for all considered models compared to CPU-based computation.

Conclusions

We implemented a GPU-based simulation framework from scratch to enable unrestricted investigation of a broad range of topics without the limitations imposed by closed source frameworks. Using the implemented simulation framework we are able to reproduce well known mechanism such as the formation of spiral waves during atrial fibrillation, low energy defibrillation, and the break up of sprial waves using a point stimulus.

Based on a high-performance simulation framework capable of computing the electrical excitation wave dynamics in realistic geometries in reasonable computation time, many scientific objectives can be addressed in future studies. For example, novel low-energy defibrillation strategies which have been developed in simple 2D settings (5) can be investigated in more realistic (patient specific) situations.

References

1. Barkley D. A model for fast computer simulation of waves in excitable media. Physica D: Nonlinear Phenomena 1991; 49:61–70
2. Fenton F and Karma A. Vortex dynamics in three-dimensional continuous myocardium with fiber rotation: Filament instability and fibrillation. Chaos: An Interdisciplinary Journal of Nonlinear Science 1998; 8:20–47
3. Bueno-Orovio A, Cherry EM, and Fenton FH. Minimal model for human ventricular action potentials in tissue. Journal of theoretical biology 2008; 253:544–60
4. Tusscher KH ten, Noble D, Noble PJ, and Panfilov AV. A model for human ventricular tissue. American Journal of Physiology-Heart and Circulatory Physiology 2004
5. Lilienkamp T, Parlitz U, and Luther S. Taming cardiac arrhythmias: Terminating spiral wave chaos by adaptive deceleration pacing. Chaos: An Interdisciplinary Journal of Nonlinear Science 2022 Dec; 32. Publisher: American Institute of Physics:121105. doi: 10.1063/5.0126682. Available from: https://aip.scitation.org/doi/10.1063/5.0126682 [Accessed on: 2023 Mar 17]

Pharmacophore Based Virtual Screening campaign to identify novel glutamate carboxypeptidase II inhibitors

Veronika Temml[1], Zsofia Kutil[2], Jakub Kollar[1], Daniela Schuster[1]

[1]Institute of Pharmacy, Department of Pharmaceutical and Medicinal Chemistry, Paracelsus Medical University, Salzburg, Austria; [2]Laboratory of Structural Biology, Institute of Biotechnology of the Czech Academy of Sciences, BIOCEV, Prumyslova 595, 252 50 Vestec, Czech Republic; Contact: Veronika.Temml@pmu.ac.at

Objective

Glutamate carboxypeptidase II (GCPII) is a metalloprotease serving as a marker in prostate oncology but is also associated with neurological pathologies. While several classes of potent GCPII-specific inhibitors exist, the development of novel active scaffolds with potential to cross the blood-brain-barrier to explore the target's neurological potential remains a challenge. [1]. The binding pocket of GCPII can be roughly divided in three subsites, the S1 and S1' site forming a cavity close to the two zinc ions in the catalytic center of binding the pocket and the AB site, formed by an Arg patch at the entry site of the cavity. Known GCPII inhibitors can be sorted by binding mode and the wealth of crystallographic data on the target allows for the development of site tailored specific pharmacophore models [2].

Methods

Pharmacophore modelling derives the known protein ligand interactions as a threedimensional pattern of physicochemical features that can then be used to search for molecules that could display a similar interaction patterns. Pharmacophore based virtual screening is a technique that allows us to select the most promising candidate molecules for experimental testing. The software Ligandscout (www.inteligand.com) was used in this study.

Results

Structure- and ligand-based pharmacophore models were created based on a dataset of known GCPII-selective ligands. These models were used in a virtual screening of the SPECS compound library (~209.000 compounds). Fifty top-scored virtual hits were further experimentally tested for their ability to inhibit GCPII enzymatic activity in vitro. Six hits were found to have moderate to high inhibitory potency with the best virtual hit, a modified xanthene, inhibiting GCPII with an IC50 value of 353±24 nM.

Conclusions

The identification of this novel inhibitory scaffold illustrates the applicability of the pharmacophore-based modeling for the discovery of GCPII-specific inhibitors.

Acknowledgements

We thank inte:ligand for providing the software ligandscout free of charge.
V.T. is funded by the FWF project T942

References

1. Tsukamoto, T et al, Progress in the Discovery and Development of Glutamate Carboxypeptidase II Inhibitors. Drug Discov. Today 2007, 12 (17–18), 767–776.
2. Temml V. et al,, Combination of In Silico and In Vitro Screening to Identify Novel Glutamate Carboxypeptidase II Inhibitors, J. Chem. Inf. Model. 2023, 63, 4, 1249–1259.

Simulation of blood flow in umbilical vessels and catheters for the artificial placenta

Maximilian Tiedemann, Ferdinand Rehme, Stanimir Georgiev, Peter Ewert, Stefan Eckstein, Christoph Fusch[1,3], Niels Rochow[1,3,4]

[1]Department of Pediatrics, Paracelsus Medical University, Nuremberg, Germany, [2]Nuremberg Institute of Technology, Germany, [3]Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada, [4]Department of Pediatrics, University Medicine Rostock, Rostock, Germany

Objective
To connect the artificial placenta requiring a large bore vascular access with the systemic circulation, umbilical vein and artery can be cannulated. A sufficient amount of blood must bypass the systemic circulation and enter the extracorporeal circuit of the artificial placenta to sustain the oxygen supply and CO2 removal of the infant. Like in the natural placenta, the blood flow is driven only by the infant's heart without pumps. Recently, an extracorporeal blood flow of 30 to 80 mL/kg/min was suggested. Therefore, it is necessary to determine the pressure drop in and the volume of the catheters. The aim of this study is to determine which diameters these catheters can have, in order to fulfill the criteria.

Methods
This simulation study analyzed the blood flow in different proposed catheters for five body weight strata (<800g; 800g to 1,249g; 1,250g to 1,999g; 2,000g to 2,999g; ≥3,000g). The size of the umbilical vessel was obtained from radiographs of 25 newborns. The diameter of umbilical vessels was obtained from published literature. The blood flow in umbilical vessels was set to fetal conditions. Novel large bore catheters with a wall diameter of 0.5 mm were proposed. Simulation was performed using Ansys Discovery. Pressure drop and dead volume was analyzed. Further, the catheter size was optimized to achieve a pressure drop of 1mmHg/kg. A volume flow of 50ml/kg/min was assumed for all models.

Results
All simulated flows were laminar. The inner diameters of the umbilical vein for the five weight strata were in ascending order of weight: 6mm; 7.6mm; 8.4mm; 8.7mm; 8.4mm. For the umbilical arteries they were: 3.4mm; 3.9mm; 4.2mm; 4.2mm; 4mm. The average pressure drop in these vessels was 0.45 ±0.25mmHg. The average dead volume is 3.1 ±0.5mL per kilogram bodyweight.
For a pressure drop of 1mmHg/kg, the minimal inner diameters of the catheters in the umbilical vein for the five weight strata were in ascending order of weight: 5.0mm; 5.6mm; 6.2mm; 6.9mm; 7.0mm. For the umbilical arteries they were: 2.4mm; 2.4mm; 2.6mm; 2.8mm; 2.8mm. The dead volumes in the minimal size catheters for the five weight strata in ascending order of weight were: 1.8mL; 2.0mL; 3.1mL; 5.2mL; 5.2mL. The average catheter dead volume is 1.8mL per kilogram bodyweight. Therefore, a volume reduction by 42% has been achieved.

Conclusions
In this study, we have determined a scope for the size of the umbilical catheters for the application of the artificial placenta. We found that the dead volume can be decreased significantly without increasing the pressure drop above the critical threshold. In further studies lower flow velocities will be examined, to achieve even lower volumes. This data will be used to develop novel large bore access umbilical catheters. The next steps in this development are finding a suitable material and determining the geometrical properties of the catheters, which prevent the catheters from folding.

Pharmacological interventions for the management of children and adolescents living with obesity – an update of a Cochrane systematic review with meta-analyses

Gabriel Torbahn[1,2], Alex Griffiths[3], Andrew Jones[4], Jamie Matu[3], Maria-Inti Metzendorf[5], Louisa Ells[3], Gerald Gartlehner[6,7], Aaron S Kelly[8], Daniel Weghuber[2,9], Tamara Brown[5]

[1]Department of Pediatrics, Paracelsus Medical University, Klinikum Nürnberg, Universitätsklinik der Paracelsus Medizinischen Privatuniversität Nürnberg, Nuremberg, Germany; [2]Department of Pediatrics, Paracelsus Medical University, Salzburg, Austria; [3]Obesity Institute, School of Health, Leeds Beckett University, Leeds, UK; [4]School of Psychology, Liverpool John Moores University, Liverpool, UK; [5]Institute of General Practice, Medical Faculty of the Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany; [6]Department for Evidence-based Medicine and Evaluation, Cochrane Austria, Danube University Krems, Austria; [7]RTI International, Research Triangle Park, North Carolina, USA; [8]Department of Pediatrics and Center for Pediatric Obesity Medicine, University of Minnesota Medical School Minneapolis, USA; [9]Department of Pediatrics, Obesity Research Unit, Paracelsus Medical University, Salzburg, Austria; Contact: gabriel.torbahn@klinikum-nuernberg.de

Objective
Anti-obesity medications (AOMs) can form an integral component of obesity treatment. A 2016 Cochrane review by Axon et al. suggested that AOMs may help reducing body mass index (BMI (kg/m2)), when delivered alongside a concomitant lifestyle intervention. New AOMs for paediatric obesity require an update of the evidence base.

Methods
We used Cochrane methodology and data from Axon et al., ran searches on 7th of September 2022 in two electronic databases (Cochrane CENTRAL, MEDLINE) and two trials registries (ClinicalTrials.gov, WHO ICTRP). We evaluated BMI, serious adverse events (sAEs), quality of life (QoL), type 2 diabetes mellitus (T2DM), mental (e.g., bullying) and social health (e.g., isolation). Where possible, we undertook pairwise random-effects meta-analyses to pool effect sizes comparing AOMs to placebo, using mean difference, or risk ratio and respective 95%-CI and investigated potential effect modifiers (e.g., age, drug type).

Results
We included 34 RCTs (4,053 participants). AOMs demonstrated a large range of differences in reduction of BMI: orlistat -0.8 [-1.1;-0.5], topiramate -0.9 [-2.2;0.5], metformin -1.3 [-1.7;-0.9], sibutramine -1.7 [-2.9;-0.5], phentermine/topiramate -4.6 [-6.2;-3.0] and for glucagon-like peptide 1 receptor agonists -1.0 [-4.2;2.2] (exenatide), -1.6 [-2.5;-0.7] (liraglutide) and -5.9 [-7.0;-4.8] (semaglutide), respectively without other effect modifiers. Overall, there was a trend for improvement in QoL (1.97 [0.2;3.8]), with no significant increase in risk for sAEs. No data were available for T2DM, mental or social health.

Conclusions
Overall, AOMs show large differences in terms of reduction in BMI, ranging from small to substantial clinical significance, and a trend for improving QoL. SAEs did not significantly differ between AOM and placebo. Missing data for T2DM, mental and social health highlight an important evidence gap in patient-relevant outcomes.

Acknowledgements
We thank the respective universities for the in kind support.

References
1. Mead E, Atkinson G, Richter B, Metzendorf MI, Baur L, Finer N, et al. Drug interventions for the treatment of obesity in children and adolescents. Cochrane Database Syst Rev. 2016 Nov 29;11(11):CD012436. doi: 10.1002/14651858.CD012436. PMID: 27899001

The diagnostic performance of sonication in differentiating septic and aseptic femoral and tibial shaft nonunion

Katharina Trenkwalder[1,2], Sandra Erichsen[1,2], Ferdinand Weisemann[3], Peter Augat[1,2], Matthias Militz[3], Simon Hackl[2,3]

[1]Institute for Biomechanics, BG Unfallklinik Murnau, Germany; [2]Institute for Biomechanics, Paracelsus Medical University Salzburg, Austria; [3]Department of Trauma Surgery, BG Unfallklinik Murnau, Germany; Contact: katharina.trenkwalder@bgu-murnau.de

Objective
In nonunion treatment, exclusion or diagnosis of an infection is of central importance, since septic and aseptic nonunion require different therapeutic strategies. However, the differential diagnosis is not always clear, as low-grade infections and biofilm bound bacteria often remain undetected by conventional diagnostics (1). Biofilm embedded bacteria on implants can be detected by sonication. In recent years, this method has become an important element in the diagnosis of prosthetic joint infection (2). But the value sonication in the diagnosis of septic nonunion has not yet been sufficiently examined. Therefore, the aim of our study was to evaluate the diagnostic performance of sonication in differentiating between septic and aseptic femoral or tibial shaft nonunion in comparison to conventional tissue culture and histopathology.

Methods
In a prospective, clinical multicenter study osteosynthesis material and tissue samples of 53 patients with aseptic, 42 with septic femoral or tibial shaft nonunion and 32 patients with regular healed fractures were obtained during nonunion revision or metal removal. Tissue samples were analyzed by conventional long-term incubation and histopathological examination. The osteosynthesis material was sonicated and the resulting sonication fluid analyzed with two different methods. It was directly broth-cultured as well as concentrated by membrane filtration followed by incubation and colony forming unit (CFU) quantification. With receiver operating characteristic (ROC) we determined CFU cut-off values for differentiation between septic and aseptic nonunion or regular healers. The diagnostic performance of the different diagnostic methods was calculated using cross tabulation.

Results
ROC curve analysis revealed an optimal cut-off value of ≥ 13.6 CFU/10 ml sonication fluid to differentiate between septic and aseptic nonunion. With a sensitivity of 52% and a specificity of 93%, the diagnostic performance of sonication with this calculated threshold was lower than that of tissue culture (69% and 96%) but higher than that of histopathology (14% and 87%). In consideration of two criteria for a confirmatory infection diagnosis, sensitivity was 55% for one tissue sample with the same germ in broth cultured sonication fluid, exactly the same as for two tissue samples with the same pathogen. The combination of tissue culture and membrane filtrated sonication fluid had a sensitivity of 50%, which increased up to 62% using a lower CFU cut-off, which was determined from the regular healers. Furthermore, membrane filtration of sonication fluid showed a significant higher detection rate of polymicrobial infections compared to tissue culture and broth cultured sonication fluid ($p<0.001$).

Conclusions
Sonication with membrane filtration is a useful additive diagnostic method that improves the differential diagnosis in nonunion, reduces the time to culture positivity and detects polymicrobial infections more frequently. These are important clinical aspects for an early initiation of bacterial-specific antibiosis.

References
1. Dapunt et al., Ther Clin Risk Manag, 2015.
2. Oliva et al., Frontiers in Microbiology, 2021.

Upregulated Inflammatory Cytokines in CSF of Multiple Sclerosis Patients

Lisa Weidner[1], Dirk Strunk[2], Christof Jungbauer[1]

[1]Austrian Red Cross, Blood Service für Vienna, Lower Austria and Burgenland, Vienna, Austria; [2]Spinal Cord Injury and Tissue Regeneration Center Salzburg, Paracelsus Medical University of Salzburg, Salzburg, Austria; Contact: lisa.weidner@stud.pmu.ac.at

Objective

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system mainly driven by Th1-cells. Current data on cytokines levels especially in cerebrospinal fluid (CSF) vary greatly depending on method and patient group. IP10 is often found in higher concentrations in patients with diseases, which involve inflammation of the brain for example MS and systemic lupus erythematodes.[1,2] In this study a bead-based cytokine analysis is used in MS patients and controls, consisting of headache or idiopathic intracranial hypotension patients, to identify differences in cytokine levels in CSF as well as in serum.

Methods

Cytokine analysis in cerebrospinal fluid and serum of 24 multiple sclerosis patients routinely drawn at first presentation and 9 controls is performed on Luminex platform with multiplex assays covering 48 different cytokines. Samples were taken from a biobank and were stored frozen.

Results

Cytokines IP10, MIG and MIP-1a are significantly ($p<0.05$) upregulated and IL-4, IL-1RA, IL-13, LIF significantly downregulated in CSF of MS patients in comparison to controls.

Conclusions

The results are compatible with a Th1-driven immune response limited to the brain. Consequently, anti-inflammatory (IL-13, IL-18) and Th2 signature cytokines (IL-4) are downregulated. With further analysis and correlation with number of T-cells and clinical symptomatic cytokine analysis in CSF during first manifestation of MS the data could help the extent of inflammation. Further cytokines in CSF are dysbalanced in MS patients in comparison to the control group.

References

1. Scarpini E, Galimberti D, Baron P, Clerici R, Ronzoni M, Conti G, Scarlato G. IP-10 and MCP-1 levels in CSF and serum from multiple sclerosis patients with different clinical subtypes of the disease. J Neurol Sci. 2002 Mar 15;195(1):41-6. doi: 10.1016/s0022-510x(01)00680-3. PMID: 11867072.
2. Yoshio T, Okamoto H, Kurasawa K, Dei Y, Hirohata S, Minota S. IL-6, IL-8, IP-10, MCP-1 and G-CSF are significantly increased in cerebrospinal fluid but not in sera of patients with central neuropsychiatric lupus erythematosus. Lupus. 2016;25(9):997-1003. doi:10.1177/0961203316629556

A Precision Medicine Tool for High Utilization and Quality of Individual Treatment Trials with Immunomodulatory Drugs in Mucopolysaccharidosis

Anna-Maria Wiesinger[1], Brian Bigger[2], Roberto Giugliani[3], Georg Zimmermann[4], Tobias Moser[5], Maurizio Scarpa[6], Christina Lampe[7], Chrtistoph Kampmann[8], Florian Lagler[1]

[1]Institute of Congenital Metabolic Diseases, Paracelsus Medical University, Salzburg, Austria; [2]Stem Cell and Neurotherapies, Division of Cell Matrix Biology and Regenerative Medicine, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK; [3]Department of Genetics, UFRGS, Medical Genetics Service and Biodiscovery Laboratory, HCPA, DASA Casa dos Raros, Porto Alegre, Brazil; [4]Team Biostatistics and Big Medical Data, IDA Lab Salzburg, Paracelsus Medical University, Salzburg, Austria; [5]Department of Neurology, Christian Doppler University Hospital, Paracelsus Medical University, Salzburg, Austria; [6]Regional Coordinating Center for Rare Diseases, University Hospital Udine, Italy; [7]Department of Child Neurology, Epilepetology and Social Pediatrics, Center of Rare Diseases, University Hospital Giessen / Marburg, Germany; [8]Department of Pediatric Cardiology, University Hospital Mainz, Mainz, Germany; Contact: anna.wiesinger@pmu.ac.at

Objective
The growing understanding of the innate immune response in Mucopolysaccharidosis (MPS) revealed potential targets for intervening, including the repurposing of immunomodulatory drugs. Individual treatment trials (ITTs) could efficiently translate this knowledge into clinical use. However, despite the limited efficacy of approved drugs and the high level of suffering this has hardly been used - at least these are not reported or published.

Methods
We analysed the subjective need for, utilisation of and barriers to ITTs by an international MPS expert survey. Based on that and by adapting validated decision analysis frameworks (DAF) we developed a benefit-risk assessment model for ITTs in MPS. Our strategy is based on the following steps (i) literature analysis on relevant targets and clinical pharmacology of immunomodulators, (ii) quantitative DAF data acquisition with the creation of a framed decision context and assigning weights to relevant outcomes, (iii) enabling personalizability by phenotypic profiling and assessing specific probabilities of beneficial outcomes, (iv) assessment standard for ITTs in MPS. These steps are in accordance with an international, interdisciplinary expert board and patient representatives.

Results
Although the majority of MPS clinicians was familiar with the concept of ITT (73%; 20/27), only few ever conducted (35%; 10/27) or published those (6%; 2/13). A free service for data-driven treatment choices is expected to increase the utilization and quality of ITTs by 89% (23/26). We identified Anakinra, Adalimumab, Abatacept and Cladribine as top candidates and defined first and second line drugs for different phenotypes.

Conclusions
Our developed evidencebased, personalizable, quantitative DAF model characterizes the first step towards precision medicine with immunomodulators in MPS.

Acknowledgements
This project has been kindly supported by MPS Austria and has been honored with the WORLDSymposium Young Investigator Award 2023.

References
1. Parker H, Bigger BW. The role of innate immunity in mucopolysaccharide diseases. J Neurochem. 2019;148(5):639-651. doi:10.1111/jnc.14632
2. van der Zanden TM, Mooij MG, Vet NJ, et al. Benefit-Risk Assessment of Off-Label Drug Use in Children: The Bravo Framework. Clin Pharmacol Ther. 2021;110(4):952-965. doi:10.1002/cpt.2336

A Decision Analysis Tool To Aid Diagnosis of ASMD Disease – Design and First Results

Anna-Maria Wiesinger[1], Eugen Mengel[2], Roberto Giugliani[3], Charlotte Aries[4], Nicole Muschol[4], Georg Zimmermann[5], Dorothea Möslinger[6], Vasiliki Konstantopoulou[6], Daniela Karall7, Sabine Scholl Bürgi[7], Florian Lagler[1]

[1]Institute of Congenital Metabolic Diseases, Paracelsus Medical University, Salzburg, Austria; [2]SphinCS GmbH, Institute of Clinical Science for LSD, Hochheim, Germany; [3]Department of Genetics, UFRGS, Medical Genetics Service and Biodiscovery Laboratory, HCPA, DASA Casa dos Raros, Porto Alegre, Brazil; [4]Department of Pediatrics, University Medical Center Hamburg, Eppendorf, Hamburg; [5]IDA Lab, Paracelsus Medical University Salzburg, Austria; [6]Department for Pediatrics and Adolescent Medicine, Inborn Errors of Metabolism, Medical University of Vienna, Vienna, Austria; [7]Department of Pediatrics, Medical University of Innsbruck, Innsbruck, Austria; Contact: anna.wiesinger@pmu.ac.at

Objective
The diagnosis of acid sphinogomyelinase deficiency (ASMD, Niemann Pick Type A, A/B, B) is frequently delayed by years, due to the highly heterogeneous and often unspecific clinical features. The involvement of different organs, the musculoskeletal system and the central nervous system represent a diagnostic challenge. Therefore, we aim to develop an innovative suspicion index tool (SIT) for health care professionals to enable an early and accurate diagnosis of ASMD.

Methods
Our SIT is based on validated tools with literature research for type specific clinical features and differential diagnosis as well as a multi-center chart review with international ASMD centers in Europe and America. ASMD symptoms were categorized into skeletal, visceral and neurologic domains. The scoring system results from the prediction of single symptoms, symptom combinations, and ASMD family history via logistic regression.

Results
To date 160 ASMD patients, non-cases and controls (M.Gaucher, M.Wolman, NPD-C) from literature and 48 further ASMD patients by expert chart review have been analyzed (ongoing). Symptoms occurring in more than one category (skeletal, visceral, neurological) as well as family relationships were strong indicators of ASMD. Visceral symptoms were highly suggestive, including splenomegaly, hepatomegaly, and mixed dyslipidemia.

Conclusions
So far, our strategy has been very feasible. After the expert chart review is finished, we will finalize the scoring system and implement it as mobile device app for evidence-based clinical support.

Acknowledgements
This project has been kindly supported by Sanofi Genzyme and has been honored with the Sanofi Medical Award.

References
1. McGovern MM, Dionisi-Vici C, Giugliani R, et al. Consensus recommendation for a diagnostic guideline for acid sphingomyelinase deficiency. Genet Med. 2017;19(9):967-974. doi:10.1038/gim.2017.7
2. Wijburg FA, Sedel F, Pineda M, et al. Development of a suspicion index to aid diagnosis of Niemann-Pick disease type C. Neurology. 2012;78(20):1560-1567. doi:10.1212/WNL.0b013e3182563b82
3. Pineda M, Mengel E, Jahnová H, et al. A Suspicion Index to aid screening of early-onset Niemann-Pick disease Type C (NP-C). BMC Pediatr. 2016;16:107. Published 2016 Jul 22. doi:10.1186/s12887-016-0641-7

Avatar- and virtual agent-assisted telecare for patients in their homes: a scoping review

Anna Winkler[1], Patrick Kutschar[1], Stefan Pitzer[1], Antje van der Zee-Neuen[1,2], Susanne Kerner[3], Simon Krutter[1], Jürgen Osterbrink[1]

[1]Paracelsus Medical University, Institute of Nursing Science and Practice, Salzburg, Austria; [2]Paracelsus Medical University, Gastein Research Institute, Salzburg, Austria; [3]Paracelsus Medical University, Bachelor´s Program Nursing Science Online, Institute of Nursing Science and Practice, Salzburg, Austria; Contact: anna.winkler@pmu.ac.at

Objective
Telecare can be an effective way to deliver healthcare to patients' homes.[1] Avatar- or virtual agent-equipped technologies have the potential to increase user engagement and adherence to telecare.[2] A part of a parental study,[3] this review aimed to identify telecare interventions assisted by avatars/virtual agents, reflect the concepts of telecare and give an overview on its outcomes.

Methods
A scoping review guided by the PRISMA-ScR checklist was conducted. MEDLINE, CINAHL, PsycINFO and grey literature were searched through 12 July 2022. Studies were included if patients were remotely cared for by healthcare professionals and received telecare interventions assisted by avatars/virtual agents in their homes. Search results were imported into Covidence™, independently screened by two reviewers and their quality assessed using JBI's critical appraisal tools. Studies were synthesized along the dimensions 'study characteristics', 'intervention', and 'outcomes'.

Results
Out of 535 records screened, 14 studies were included. Telecare interventions focused on teletherapy (n=11), telemonitoring (n=9), teleconsultation (n=6) and telelearning (n=4). The telecare services invoked were rehabilitative (n=8), preventive (n=4), palliative (n=3), promotive (n=3) and curative (n=1). Modes of communication were asynchronous only (n=6), synchronous only (n=4) or both (n=4). Tasks of the implemented avatars/virtual agents comprised delivering health interventions (n=8), guidance and strengthening agency (n=4) as well as monitoring and taking assessments (n=2). Telecare interventions led to improved clinical outcomes and higher adherence. Most studies reported sufficient system usability and high satisfaction among participants.

Conclusions
Telecare interventions were overall target group related and integrated in a service model. This combined with the use of avatars and virtual agents leads to improved adherence to telecare in the home setting. Further studies could account for relatives' experiences with telecare.

Acknowledgements
This work is supported by Electronic Caregiver Inc., Las Cruces, New Mexico, USA (no grant number).

References
1. Sanchez-Ramirez DC, Pol M, Loewen H, Choukou M-A. Effect of telemonitoring and telerehabilitation on physical activity, exercise capacity, health-related quality of life and healthcare use in patients with chronic lung diseases or COVID-19: a scoping review. J Telemed Telecare. 2022;0(0):1357633X221122124.
2. Wonggom P, Kourbelis C, Newman P, Du H, Clark RA. Effectiveness of avatar-based technology in patient education for improving chronic disease knowledge and self-care behavior: a systematic review. JBI evid. synth. 2019;17(6).
3. Krutter S, Schuessler N, Kutschar P, et al. Piloting of the virtual telecare technology 'Addison Care' to promote self-management in persons with chronic diseases in a community setting: protocol for a mixed-methods user experience, user engagement and usability pilot study. BMJ Open. 2022;12:e062159.

Sensitivity of a Fully Automated Image Analysis Pipeline to Transverse (T2) Relaxation Time Differences of Articular Cartilage in Knees with and without Cartilage Damage - On Behalf of the OA-BIO Consortium

Anna Wisser[1], Frank Roemer[2,3], Francis Berenbaum[4,5,6], Jana Kemnitz[1], Susanne Maschek[1], Felix Eckstein[1], Wolfgang Wirth[1]

[1]Dept. of Imaging & Functional Musculoskeletal Res., Institute of Anatomy & Cell Biology, Paracelsus Med. Univ., Salzburg, Austria; [2]Radiology Dept., Univ. of Erlangen; [3]Boston Univ., Boston, MA; [4]AP-HP Saint-Antoine Hosp., Paris, France; [5]Sorbonne Univ., Paris, France; [6]4Moving Biotech, Lille, France; Contact: anna.wisser@pmu.ac.at

Objective
MRI-detected cartilage damage is associated with elevated cartilage T2 relaxation times in MRI. Further, cartilage damage is more frequently observed in knees at higher risk of developing radiographic knee osteoarthritis (OA). The aim of this study was to compare a U-Net-based, fully automated cartilage segmentation pipeline vs. manual, quality-controlled cartilage segmentations for determining laminar cartilage T2 differences between KLG0 knees with vs. without cartilage damage (Funding: OA-BIO Eurostars-2 project, E! 114932).

Methods
The fully automated U-Net-based image analysis pipeline was trained using sagittal multi-echo spin-echo (MESE) MRIs from the Osteoarthritis Initiative (OAI) [1]. U-Nets for medial (MFTC) and lateral (LFTC) femorotibial cartilage segmentation were trained on all seven echoes of the MESE MRIs of 92 OAI healthy reference cohort (HRC) participants. A third U-Net was trained on bone segmentations of 60 OAI HRC knees. The bone segmentation served for determining the weight-bearing femoral region of interest and for selecting the MRI slices that require cartilage segmentation. MFTC and LFTC U-Nets were then applied to the selected MRI slices (OAI incidence cohort) of 123 KLG0 knees (small sample) with, and 618 KLG0 knees without manual segmentations available (total of 741 knees = full sample). Automated post-processing was used to identify and correct implausible segmentations. Differences in superficial and deep layer cartilage T2 obtained from U-Net and manual segmentations were compared for knees with vs. without MRI Osteoarthritis Knee Score (MOAKS) cartilage damage in the small and full sample. MOAKS readings were performed by a very experienced MSK radiologist. Effect sizes were measured using Cohen's D (d).

Results
The full sample comprised knees of 415 women and 326 men (age: 60y, BMI: 27 kg/m²). Of these, 408 had no cartilage damage, 115 only medial damage, 149 only lateral damage, 69 had medial and lateral damage. In the MFTC, deep layer T2 did not differ between knees with vs. without cartilage damage for both U-Net and manual cartilage segmentations, in both samples (d: 0.04 to 0.09). Yet, superficial layer T2 was longer in knees with cartilage damage than in those without (d [95% CI] 0.63 [0.26, 0.99]) for manual segmentations and 0.58 [0.22, 0.95] for U-Net segmentations in the small sample; in the full sample it was 0.70 [0.53, 0.87]). Manual cartilage segmentations of the LFTC yielded greater T2 for both the deep (d: 0.48 [0.12, 0.85]) and superficial layers (d: 0.66 [0.29, 1.03]) in knees with vs. without cartilage damage. Results for U-Net segmentations were 0.59 [0.22, 0.95] (small sample) and 0.43 [0.27, 0.59] (full sample) for the deep layer and 0.66 [0.29, 1.02] (small sample) and 0.59 [0.43, 0.75] (full sample) for the superficial layer.

Conclusions
The fully automated U-Net-based cartilage segmentation pipeline was at least as sensitive to differences in laminar cartilage T2 between KLG 0 knees with vs. without MOAKS cartilage damage as quality-controlled, manual cartilage segmentation. Hence this approach shows great potential for laminar cartilage T2 analyses and can be applied to large samples.

References
1. Wirth et al (2019), Osteoarthritis and Cartilage. 27(11):1663-1668

Designing a protein corona around extracellular vesicles for angiogenesis and wound healing

Martin Wolf[1], Rodolphe Poupardin[1], Patricia Ebner-Peking[1], Andre Cronemberger Andrade[1], Constantin Blöchl[2], Astrid Obermayer[2], Fausto Gueths Gomes[1,3], Balazs Vari[1], Essi Eminger[1], Heide-Marie Binder[1], Anna M. Raninger[1], Sarah Hochmann[1], Gabriele Brachtl[1], Andreas Spittler[4], Thomas Heuser[5], Racheli Ofir[6], Christian G Huber, Zami Aberman[6], Katharina Schallmoser[3], Hans-Dieter Volk[7], Dirk Strunk[7]

[1]Cell Therapy Institute, Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University (PMU), Salzburg, Austria; [2]Department of Biosciences, Paris Lodron University Salzburg; [3]Department of Transfusion Medicine and SCI-TReCS, PMU, Salzburg, Austria; [4]Anna Spiegel Center of Translational Research, Medical University, Vienna; [5]Vienna Biocenter Core Facilities, Medical University, Vienna; [6]Pluristem Ltd., Haifa, Israel; [7]BCRT & Institute of Medical Immunology, Charite - Universitätsmedizin Berlin, Germany; Contact: martin.wolf@pmu.ac.at

Objective

Recently evidence was accumulating that extracellular vesicles (EVs) can carry a protein corona[1,2] around them similar as it was reported for synthetic nanoparticles. As we could show that EVs derived from therapeutic grade placental stroma cells (PLX) contribute to their efficacy in aiding re-vascularization of ischemic tissue we wanted to investigate whether a protein corona around these vesicles contributes to this effect.

Methods

EVs were enriched from PLX conditioned media via tangential flow filtration (TFF), followed by size exclusion or ultracentrifugation. Identity and purity of the different EV preparations was analysed by western blot (WB), super resolution microscopy and transmission electron microscopy (TEM). To analyse their functionality we utilized in vitro angiogenesis assay, stimulation capacity of Immune cells, and in vivo wound healing mouse model. A proteomis approach was used to identify active components in the EV fractions. Finally we tried to re-establish a functional protein corona with defined factors around naked EVs.

Results

EV identity according to the MISEV criteria was proven via WB. With super resolution microscopy we found a high degree of heterogeneity of ttetraspanin (CD9, CD63 and CD81) expression on a single EV level. PLX-EVs purified via TFF displayed proangiogenic potential and inhibition of T cell proliferation in vitro as well as enhanced vessel density and wound healing in an in vivo mouse model. When further purified via ultracentrifugation or SEC, EVs lost these functions. This effect was accompanied by a loss of their protein corona visualized by negative contrast TEM imaging. When re-estblishing the protein corona with defined factors their function could be restored.

Conclusions

We found that EVs from PLX cells purified via TFF carry a protein corona around them. Although removal of the natural protein corona resulted in a loss of their function replacing it with defined factors restored their function.

References

1. Tóth, E. Á. et al. Formation of a protein corona on the surface of extracellular vesicles in blood plasma. J. Extracell. Vesicles 10, e12140 (2021).
2. Wolf M. et al. A functional corona around extracellular vesicles enhances angiogenesis, skin regeneration and immunomodulation. J Extracell Vesicles. 2022 Apr; 11(4): e12207.

Genetic variants determine treatment response in autoimmune hepatitis

Stephan Zandanell[1], Lorenz Balcar[2], Georg Semmler[2], Isabella Leitner[3], Alex Schirmer[1], Lea Rosenstatter[1], David Niederseer[4], Karl Sotlar[5], Anna-Maria Schneider[6], Michael Strasser[1], Sophie Gensluckner[1], Alexandra Feldman[1], Christian Datz[3], Bernhard Paulweber[1], Elmar Aigner[1]

[1]First Department of Medicine, Paracelsus Medical University, Salzburg, Austria; [2]Division of Gastroenterology and Hepatology, Department of Internal Medicine III, Medical University of Vienna, Austria; [3]Department of Internal Medicine, Oberndorf Hospital, Oberndorf, Austria; [4]Department of Otorhinolaryngology, Cantonal Hospital Aarau, Switzerland; [5]Department of Cardiology, University Heart Center Zürich, Zürich, Switzerland; [6]Institute of Pathology, Paracelsus Medical University, Salzburg, Austria; Contact: S.zandanell@salk.at

Objective
Autoimmune hepatitis (AIH) is a rare entity; in addition, single-nucleotide polymorphisms (SNPs) may impact its course and outcome. We investigated the role of liver-related SNPs regarding activity, stage and treatment response in a Central European AIH cohort.

Methods
A total of 113 AIH patients (i.e., 30 male/83 female, median 57.9 years) were identified. In 81, genotyping of PNPLA3-rs738409, MBOAT7-rs626238, TM6SF2-rs58542926, and HSD17B13-rs72613567:TA, as well as both biochemical and clinical data at baseline and follow-up, were available.

Results
The median time of follow-up was 2.8 years; five patients died and one underwent liver transplantation. PNPLA3-G/G homozygosity was linked to a worse treatment response when compared to wildtype [wt] (ALT 1.7 vs. 0.6 × ULN, $p < 0.001$). MBOAT7-C/C homozygosity was linked to non-response vs. wt and heterozygosity ($p = 0.022$). Male gender was associated with non-response (OR 14.5, $p = 0.012$) and a higher prevalence of PNPLA3 (G/G vs. C/G vs. wt 41.9/40.0/15.0% males, $p = 0.03$). The MBOAT7 wt was linked to less histological fibrosis ($p = 0.008$), while no effects for other SNPs were noted. A polygenic risk score was utilized comprising all SNPs and correlated with the treatment response ($p = 0.04$)

Conclusions
Our data suggest that genetic risk variants impact the treatment response of AIH in a gene-dosage-dependent manner. Furthermore, MBOAT7 and PNPLA3 mediated most of the observed effects, the latter explaining, in part, the predisposition of male subjects to worse treatment responses.

References
1. Gronbaek, L.; Vilstrup, H.; Jepsen, P. Autoimmune hepatitis in Denmark: Incidence, prevalence, prognosis, and causes of death. A nationwide registry-based cohort study. J. Hepatol. 2014, 60, 612–617
2. Keating, J.J.; O'Brien, C.J.; Stellon, A.J; Portmann, B.C.; Johnson, R.D.; Johnson, P.J.; Williams, R. Influence of aetiology, clinical and histological features on survival in chronic active hepatitis: An analysis of 204 patients. Q. J. Med. 1987, 62, 59–66

CXCL16-CXCR6 AXIS INVOLVEMENT IN ALZHEIMER'S DISEASE-SPECIFIC CD8+T-CELLS RECRUITMENT TO THE BRAIN

Marco Zattoni[1,2], Rodolphe Poupardin[2,3], Sofia Weinbender[1,2], Sabine Bernegger[1,2], Heike Mrowetz[1,2], Barbara Altendorfer[1,2], Ludwig Aigner[1,2,4]

[1]Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg, Austria; [2]Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University, Salzburg, Austria; [3]Institute of Experimental and Clinical Cell Therapy, Paracelsus Medical University, Salzburg, Austria; [4]Austrian Cluster for Tissue Regeneration; Contact: marco.zattoni@pmu.ac.at

Objective
Together with astrogliosis and microglia activation, Alzheimer´s disease (AD) progression has been recently associated with infiltration of $CD8^+$T-cells into disease-affected brain parenchyma (1). However, the mechanism involved in $CD8^+$T-accumulation in the brain tissue remains unknown. Single cell RNA (scRNA) sequencing analysis on AD- and virus-affected brains suggested a potential role of the Cxcl16-Cxcr6 axis in the recruitment of $CD8^+$T-cells (2, 3). Our recent molecular analysis on brain-isolated $CD3^+CD8^+$T-cells revealed higher abundance of Cxcr6 in APP/PS1 animals compared to controls (4).

Methods
We take advantage of an open source scRNA sequencing dataset of brain-derived $CD45^+$cells (https://www.brainimmuneatlas.org/index.php) to identify the cellular source of Cxcl16 and Cxcr6 and we perform quantitative PCR (qPCR) and immunohistochemical analysis using brain tissue from microglia-depleted mice (5).

Results
Automated cell type annotation and t-distributed stochastic neighbour embedding analysis reveal that Cxcr6 is mainly expressed by $CD8^+$T-cells, while Cxcl16 expression is characteristic of brain macrophages (*i.e.* microglial cells). qPCR analysis indicates a significant overexpression of Cxcl16 and Cxcr6 in samples from the diseased mouse model and reduced expression levels of both Cxcl16 and Cxcr6 (observed also using immunohistochemistry) in microglia-depleted APP/PS1 mice compared to control animals

Conclusions
Overall, these results suggest putative involvement Cxcl16-Cxcr6 axis in the recruitment of $CD8^+$T-cells in an AD mouse model. Further in vitro and in vivo functional analysis may pave the way for the identification of other dysregulated molecular pathways involved in Alzheimer´s and other related neurodegenerative diseases

Acknowledgements
This work is supported by the FWF grant (P 35417-B) "Defining the role of $CD8^+$T-cells in Alzheimer's disease", PMU-FFF Stand-Alone-Project "Molecular profiling of $CD8^+$T-cells in Alzheimer's disease: new immune mediators in the brain?" (E-20/32/169-UNG)

References
1. Gate D, Saligrama N, Leventhal O, Yang AC, Unger MS, Middeldorp J, et al. Clonally expanded CD8 T cells patrol the cerebrospinal fluid in Alzheimer's disease. Nature. 2020;577(7790):399-404
2. Piehl N, rain aging and cognitive impairment. Cell. 2022;185(26):5028-39.e13
3. Rosen SF, Soung AL, Yang W, Ai S, Kanmogne M, Davé VA, et al. Single-cell RNA transcriptome analysis of CNS immune cells reveals CXCL16/CXCR6 as maintenance factors for tissue-resident T cells that drive synapse elimination. Genome Med. 2022;14(1):108
4. Altendorfer B, Unger MS, Poupardin R, Hoog A, Asslaber D, Gratz IK, et al. Transcriptomic Profiling Identifies CD8 T Cells in the Brain of Aged and Alzheimer's Disease Transgenic Mice as Tissue-Resident Memory T Cells. J Immunol. 2022;209(7):1272-85
5. Unger MS, Schernthaner P, Marschallinger J, Mrowetz H, Aigner L. Microglia prevent peripheral immune cell invasion and promote an anti-inflammatory environment in the brain of APP-PS1 transgenic mice. J Neuroinflammation. 2018;15(1):274

DOPAMINE RECEPTOR LIGAND SELECTIVITY – AN IN SILICO / IN VITRO INSIGHT

Lukas Zell[1], Alina Bretl[1], Veronika Temml[1], Daniela Schuster[1]

[1]Institute of Pharmacy, Department of Pharmaceutical and Medicinal Chemistry, Paracelsus Medical University, Salzburg, Austria; Contact: lukas.zell@pmu.ac.at

Objective

Different dopamine receptor (DR) subtypes are involved in pathophysiological conditions such as Parkinson´s Disease (PD), schizophrenia, and depression. While many DR-targeting drugs have been approved by the FDA, only a very small number is truly selective for one of the DR subtypes. Additionally, most of them show promiscuous activity at related G-protein coupled receptors, thus, suffering from diverse side-effect profiles. Different studies have shown that combined in silico / in vitro approaches are beneficial in drug discovery processes. They are, however, rarely applied to divulge the mechanisms behind ligand selectivity.

Methods

In vitro binding affinity of the investigated ligands was assessed using a cell-based HTRF assay allowing for comparing KI values of D1R, D2R, and D3R. In silico methods included a molecular docking workflow and a distance-based approach comparing binding poses within DR subtype binding pockets. Docking was performed in GOLD and visualized in LigandScout to identify the most frequent pose. The distance-based approach utilized DiscoveryStudio to calculate distances. Eventually, in vitro and in silico results were correlated calculating fold-differences, subsequently generating a scatter plot.

Results

In this study, novel DR ligands were investigated in vitro to assess binding affinities at different DR subtypes. Thus, nine D2like-selective ligands exerting micro- to nanomolar binding affinities were successfully identified. All ligands showed the highest binding affinities at the D3R. The most promising ligand exerted nanomolar D3R activity (KI = 2.3 nM) with 263.7-fold D2R/D3R selectivity. Subsequently, ligands were analyzed using a molecular docking workflow to retrospectively investigate ligand selectivity based on ligand interaction with a secondary binding pocket, supporting selectivity data determined in vitro (workflow was validated with DR ligand datasets extracted from ChEMBL). The comparison of in silico and in vitro data highlighted the potential of the developed workflow to identify compounds preferably binding to D2R and D3R (D2like-selective ligands).

Conclusions

The developed workflow and identified ligands could aid in further understanding structural motifs responsible for DR subtype selectivity, thus, aiding drug development in pathologies associated with D2R and D3R (D2like receptors) like PD. Moreover, the workflow performs well independent of the chemical scaffold of the investigated compounds, thus, covering a large chemical space. In summary, the investigated ligands as well as the developed workflow itself represent a valuable tool to investigate DR ligand selectivity and elaborate upon mechanisms driving selectivity.

References

1. Azam, S., et al.; G-Protein-Coupled Receptors in CNS: A Potential Therapeutic Target for Intervention in Neurodegenerative Disorders and Associated Cognitive Deficits. Cells, 2020. 9(2).
2. Hauser, A.S., et al.; Pharmacogenomics of GPCR Drug Targets. Cell, 2018. 172(1-2): p. 41-54 e19.
3. Sharma, V.W., S.; Kumar, H.; Structure- and ligand-based drug design: concepts, approaches, and challenges, in Chemoinformatics and Bioinformatics in the Pharmaceutical Sciences. Elsevier, 2021. p. 27-53
4. Zell, L.; Kollár, J.; Lainer, C.; Temml, V.; Schuster, D.; Identification of Novel Dopamine D2 Receptor Ligands – A Combined In Silico / In Vitro Approach. Molecules, 2022. (27)14:4435.

Microbiologic diagnostics and pathogen spectrum in infective endocarditis of surgically treated patients: a five-year, retrospective, monocentric study including 224 cases

Renate Ziegler[1], Hendrik Arnold[1,2], Ralph Bertram[1], Walter Geißdörfer[3], Matthias Pauschinger[4], Theodor Fischlein[5], Francesco Pollari[5], Joerg Steinmann[1]

[1]Institute of Clinical Hygiene, Medical Microbiology and Infectiology, Paracelsus Medical University, Klinikum Nürnberg, Nuremberg, Germany; [2]Department of Hematology and Medical Oncology, Paracelsus Medical University, Klinikum Nürnberg, Nuremberg, Germany; [3]Mikrobiologisches Institut - Klinische Mikrobiologie, Immunologie und Hygiene, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany; [4]Department of Cardiology, Paracelsus Medical University, Klinikum Nürnberg, Nuremberg, Germany; [5]Department of Cardiac Surgery, Cardiovascular Center, Paracelsus Medical University Nuremberg, Klinikum Nürnberg, Nuremberg, Germany; Contact: renate.ziegler@klinikum-nuernberg.de

Objective
The spectrum of causative organisms in infective endocarditis (IE) has changed significantly in the last decades. Reliable pathogen detection is crucial for appropriate antimicrobial therapy for IE. The aim of the study was to evaluate the diagnostic value of microbiological methods for detecting the causative microorganism of IE and to analyse the spectrum of pathogens.

Methods
A total of 224 cases (211 unique patients, some with multiple surgeries) were included into this retrospective study. Patients were diagnosed with IE according to the modified Duke criteria from January 2016 to July 2021 and underwent heart valve surgery in a tertiary hospital. Pathogen detection was performed by blood culture, microbiological culture and 16S rDNA PCR of explanted heart valve material.

Results
A causative pathogen of IE was detected in 95.5% (n = 214) of cases. Blood cultures were positive in 83.3%, while a pathogen in the examined heart valve samples was identified in 32.6% by culture and in 88.2% by 16S rDNA PCR. A microorganism was identified by 16S rDNA PCR in 61.1% of blood culture negative cases but only in 19.4% by heart valve culture. The most common pathogens were *Staphylococcus aureus* (27%), viridans group streptococci (20%), enterococci (19%) and coagulase-negative staphylococci (CoNS 8%). *Cutibacterium acnes* (7%) was detected in prosthetic valve IE cases only.

Conclusions
Blood culture as a comparatively non-invasive and straightforward technique remains an important and reliable method for initial detection of the causative organism in IE. Diagnostic stewardship programs should broadly emphasize proper collection of blood cultures, particularly sampling prior to any antibiotic treatment. Additionally, molecular testing using 16S rDNA tissue PCR can be used with culture techniques to increase the diagnostic yield, especially in the case of a negative blood culture.

Acknowledgements
We would like to thank the laboratory staff for their contributions to this study.

References
1. -

A novel fluorescence-based screen of gene editing molecules for junctional epidermolysis bullosa

Janine Zwicklhuber[1], Thomas Kocher[1], Bernadette Liemberger[1], Stefan Hainzl[1], Johannes Bischof[1], Dirk Strunk[2], Anna M. Raninger[2], Iris Gratz[1,3], Verena Wally[1], Christina Guttmann-Gruber[1], Josefina Piñón Hofbauer[1], Johann W. Bauer[4], Ulrich Koller[1]

[1]EB House Austria, Department of Dermatology and Allergology, University Hospital of the Paracelsus Medical University, Salzburg, Austria; [2]Cell Therapy Institute, Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University, Salzburg, Austria; [3]Department of Biosciences and Medical Biology, University of Salzburg, Salzburg, Austria; [4]Department of Dermatology and Allergology, University Hospital of the Paracelsus Medical University, Salzburg, Austria; Contact: j.zwicklhuber@salk.at

Objective
Junctional epidermolysis bullosa (JEB) is a severe blistering skin disease caused by mutations in the genes encoding proteins essential for skin integrity. In this study, we developed a cell line suitable for gene expression studies of the JEB-associated *COL17A1* encoding type XVII collagen (C17), a transmembrane protein involved in connecting basal keratinocytes to the underlying dermis of the skin. Designer nucleases, such as CRISPR/Cas9, represent promising tools for the permanent correction of disease-associated *COL17A1* mutations in a site-specific manner. Therefore, we developed an easy-to-use and fast applicable screening cell line to analyze the repair of *COL17A1* via CRISPR/Cas9.

Methods
Using the CRISPR/Cas9 system of *Streptococcus pyogenes* we fused the coding sequence of GFP to *COL17A1* leading to the constitutive expression of GFP-C17 fusion proteins under control of the endogenous promoter in human wild-type and JEB keratinocytes. Correct genomic integration of GFP between the *COL17A1* promotor and the *COL17A1* gene, was analyzed using PCR analysis on genomic level. Full-length expression and localization of GFP-C17 to the plasma membrane was studied via Western blot analysis and fluorescence microscopy. Furthermore, localization of the restored fusion protein was analyzed in 3D skin equivalents.

Results
The comparison of GFP-C17 and wild-type C17 by immunofluorescence staining revealed the correct localization of the fusion protein in the plasma membrane in wild-type keratinocytes. As expected, the expression of GFP-C17^{mut} fusion proteins in JEB keratinocytes generated no specific GFP signal. However, the CRISPR/Cas9-mediated repair of a JEB-associated frameshift mutation in GFP-*COL17A1*^{mut}-expressing JEB cells led to the restoration of GFP-C17, apparent in the full-length expression of the fusion protein, its accurate localization within the plasma membrane of keratinocyte monolayers, as well as within the basement membrane zone of 3D-skin equivalents.

Conclusions
In this study, we generated a reporter cell line that would be suitable for the selection and comparison of gene editing strategies/molecules for junctional EB. Currently, it is necessary to conduct time- and resource-intensive immunofluorescence stainings on fixed keratinocytes in order to evaluate the expression and localization of C17 within JEB keratinocytes. By using our novel GFP-C17 cell line we are now able to perform live cell imaging directly after treatment, thereby facilitating and accelerating the analysis of new gene editing platforms. Thus, this fluorescence-based JEB cell line provides the potential to serve as a platform to screen for personalised gene editing molecules and applications *in vitro* and in appropriate animal models *in vivo*.

References
1. Zwicklhuber J. et al.; A novel fluorescence-based screen of gene editing molecules for junctional epidermolysis bullosa. Int. J. Mol. Sci. 2023; PMID: 36982270; DOI: 10.3390/ijms24065197

Liste der Einrichtungen (korrespondierende Autor*innen)

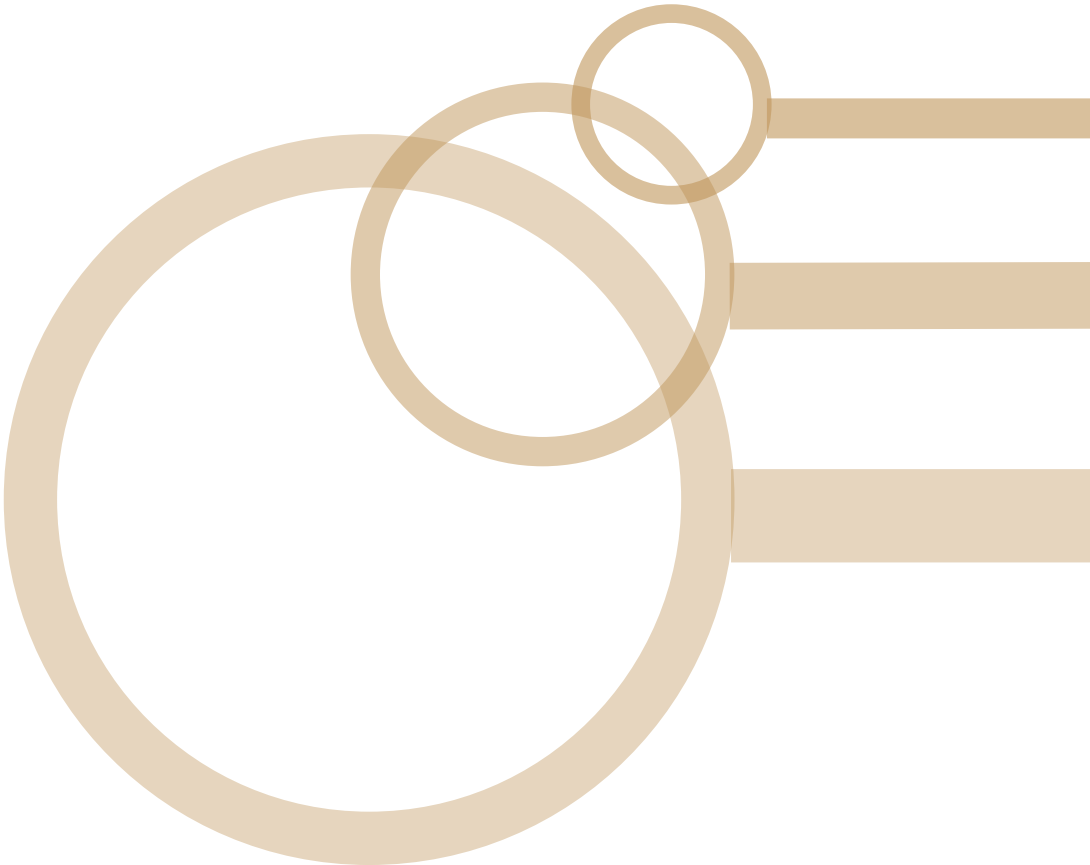
(in alphabetischer Reihenfolge)

- Berufsgenossenschaftliche Unfallklinik Murnau – Lehrkrankenhaus der PMU Salzburg
- Fachhochschule Salzburg
- Ludwig Boltzmann Institut für digitale Gesundheit und Prävention
- Paracelsus Medizinische Privatuniversität Nürnberg / Klinikum Nürnberg
- Paracelsus Medizinische Privatuniversität Salzburg / Universitätsklinikum Salzburg
- Südtiroler Sanitätsbetrieb
- Technischen Hochschule Nürnberg Georg Simon Ohm
- Technische Universität München
- Università degli Studi di Messina (Universität Messina)

Verzeichnis aller Autor*innen

(fett gedruckt = Erstautor*in)

#vSGT



Paracelsus Medizinische Privatuniversität
Forschungsmanagement | Salzburg
Strubergasse 21, 5020 Salzburg, Austria
+43 (0)662 2420-80281
www.pmu.ac.at

ISBN: 978-3-9505441-0-7