Communication **Open Access**

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38th International Winter-Workshop Clinical, Chemical and Biochemical Aspects of Pteridines and Related Topics Innsbruck, February 26th – March 1st, 2019

https://doi.org/10.1515/pteridines-2019-0010
received April 26, 2019; accepted April 26, 2019.

Neuroimaging in psychiatry and tryptophan-kynurenine metabolism

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The tryptophan metabolism has been associated with bipolar disorder (BD) in several (diverse) lines of evidence. Even though it is well known that kynurenic acid has inhibitory effects on NMDA-receptor signaling and therefore holds neuroprotective properties, the kynurenine pathway has not yet been investigated in depths in the field of brain imaging. Thus, we analyze the putative correlation between tryptophan metabolites and brain structure volumes, which are associated with mood regulation (amygdala and hypothalamus).

Cerebral MRI 3 tesla scans were performed in study participants with BD within the BIPFAT study. Metabolites of the tryptophan metabolism (kynurenine, kynurenic acid and Kyn/Trp ratio) were measured with ELISA in the serum of the mentioned study participants. Pearson correlation analysis was performed in SPSS (version 25). Male and female study participants are known to differ significantly in brain volumes and structures, therefore we investigated the correlation between tryptophan metabolites and brain structure volumes within females and male participants separately.

Female study participants with BD (n= 34) showed a significant correlation between the corrected hypothalamus volume and kynurenine (r= 0.416, p= 0.014) and the Kyn/Trp ratio (r= 0.404, p= 0.018), but not kynurenic acid (r= 0.005, p= 0.975). Male study participants with BD (n= 29) presented no significant correlations between kynurenine (r= 0.045, p= 0.815), Kyn/Trp ratio (r= 0.047, p= 0.809), kynurenic acid (r= 0.013, p= 0.935) and the hypothalamus volume.

Additionally to that, correlations between kynurenine, kynurenic acid, Kyn/Trp ratio and left/right amygdala volume showed sex-related differences. Female study participants with BD showed a significant correlation between right amygdala volume and kynurenine (r= -0.322, p= 0.040), but no significant correlation between kynurenine and left amygdala volume (r= -0.090, p= 0.576). There was also no significant correlation between kynurenic acid and Kyn/Trp ratio and left/right amygdala volume (p>0.05). Male study patients with BD presented no significant correlation between kynurenine parameters and left/right amygdala volume.

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In women, kynurenine and the Kyn/Trp ratio were associated with hypothalamus volume, the latter partly regulates mood via the 24h clock and the HPA axis. Furthermore, the negative correlation between kynurenine and amygdala volume is interesting, since amygdala volume enlargement in BD patients is more consistently reported than amygdala volume reduction in the literature. This result may also bring new insights for the effect of kynurenine in women with BD as the amygdala is known to be involved in emotion recognition and regulation. Nevertheless, it is still unclear whether the macroscopic volume of the hypothalamus or the amygdala has functional effects. Further functional analyses are therefore necessary.

Regulation of arginase 1 and inducible nitric oxide synthase in *Salmonella Typhimurium* infected bone marrow derived macrophages

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Interferon gamma (IFNγ) and tumor necrosis factor alpha (TNFα) are potent inducers of inducible nitric oxide (NO) synthase (iNOS) which is central for the control of *Salmonella enterica* serovar *Typhimurium (S. Typhimurium)* by macrophages. Two types of macrophages, inflammatory M1 macrophages expressing iNOS and anti-inflammatory M2 macrophages expressing arginase1 (Arg1), are involved in the immune response against *S. Typhimurium*. High expression of Arg1, which cleaves L-arginine, the substrate of iNOS, impairs host control of infection with intracellular microbes. Herein, we investigated the protective effects of TNFα and IFNγ on iNOS and Arg1 expression in macrophages and how this impacts on the immune control of *Salmonella* infection *in vitro*.
Bone marrow derived macrophages (BMDMs) generated from C57BL/6 WT mice upon M-CSF stimulation for six days were infected with *S. Typhimurium* for one hour. Cells were then stimulated with either Interleukin-4 (IL-4), IFNγ, or TNFα and combinations thereof for various time points. mRNA expression of Arg1 and iNOS was analyzed by quantitative reverse transcription polymerase chain reaction (qRT-PCR). Furthermore, bacterial load of infected BMDMs was analyzed by plating lysates on LB agar plates for determining colony forming units.

We observed that IL-4 is a potent inducer of Arg1 expression in a time dependent manner, which is more pronounced in infected than in uninfected BMDMs. Accordingly, inducible Arg1 expression was significantly suppressed by IFNγ whereas TNFα had only little effect in *S. Typhimurium* infected BMDMs. In a comparable fashion IFNγ was the most potent inducer of iNOS expression as compared to TNFα, and IL-4 blocked the latter effect more potently. Surprisingly, high iNOS expression did not translate into improved control of intracellular *Salmonella* proliferation and IL-4 stimulation even resulted in reduction of bacterial numbers.

Our data underline the inhibitory effects of IFNγ and TNFα on the IL-4 dependent Arg1 expression, leading to an enhanced generation of NO by iNOS *in vitro*. Whereas, IL-4 stimulation leads to an increase of Arg1 expression, however, to a decrease of NO levels. Ongoing experiments aim to investigate how these cytokines influence the transcriptional and epigenetic regulation of iNOS and Arg1.

Alternative molybdenum cofactor models: Synthesis of pterin-inspired dithiolene complexes of molybdenum and rhenium

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Molybdenum and tungsten cofactor-dependent oxidoreductases are physiologically essential enzymes, which are present in almost every life-form (from microorganisms to humans) and play a central role in the sulfur, nitrogen and carbon metabolism [1, 2]. In the early 1980s Rajagopalan discovered and characterized the natural molybdopterin ligand (mpt) in the active sites of the molybdenum and tungsten cofactor-dependent enzymes [3, 4]. Mpt is a higly conserved organic ligand which contains a pterin moiety, pyran ring, phosphate-group and coordinates the central metal over a dithiolene-function.
The aim of this project is to investigate the role of the pterin unit in the natural cofactor. We also focus on the influence of the molybdenum center on the cofactor´s

stability and catalytic activity. Therefore this project targets on the development of pterin-based dithiolene complexes of molybdenum but also of rhenium, that able to mimic the catalytic activity of the natural cofactor. In the search for a structural and functional moco-model, with optimum balance between stability and catalytic activity, the physical and chemical properties of the synthesized complexes are studied with particular focus on the influence of the pterin moiety and on the analogy and differences of molybdenum and rhenium.

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Exploring the role of airborne transmission of *Toxoplasma gondii* in a high seroprevalence area

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Infection with *Toxoplasma gondii (T. gondii)* has been well-known to be associated with food-borne illness acutely and it has the capability of chronically infecting the immunocompetent hosts, possibly leading to neuropsychiatric symptomatology. Additionally, in immunocompromised hosts, *T. gondii* may reactivate and lead to severe consequences. Furthermore, fetuses of pregnant women are at an increased risk of developing congenital malformations if the mother usually acquires infection during the gestation period. Certain regions have a high seroprevalence of *T. gondii* infection, which, in addition to the well-known oral ingestion of tissue cysts and oocysts, may also result from possible air-borne transmission of this parasite; a hypothesis that was recently tested in a study by Lass et al. [1]. Even though we became aware of this study after we had published our results, we had similarly hypothesized that the agricultural dust in certain high prevalence areas may, in part, contribute to the air-borne transmission of this parasite via the oocysts contained in it, which may be eventually ingested by individuals exposed to this dust.
Collection of environmental samples was done in Lancaster, Pennsylvania, an area primarily inhabited by the Old Order Amish, who also have a high seroprevalence for *T. gondii* infection. Utilizing respiratory precautions, we collected three different kinds of samples in our study: 1) swabs of settled dust from buildings in the area; 2) filters from a powerful vacuum cleaner that was used to suck agricultural dust; and 3) air-filters from air-conditioning units. Air-filters, as well as swabs of settled dust, were shredded and were subsequently fed to 37 uninfected pigs to see if they seroconverted by conducting ELISAs biweekly. After 60 days post-infection, heart tissue from each sacrificed seroconverted pig was then inoculated into 10 mice each, which were then examined for the presence of *T. gondii* tissue cysts in their brain smears. Additionally, using PCR amplification, all the samples were also examined for the presence of *T. gondii* DNA.

Seroconversion was only observed in one pig; however, subsequent validation of infection with *T. gondii* by inoculation of the heart tissue from this pig into mice did not demonstrate any evidence of infection with this parasite. Moreover, no sample showed any evidence of the presence of *T. gondii* DNA upon PCR amplification.

We did not find any evidence of air-borne transmission of *T. gondii* in the environmental samples collected by us. Our results are contrary to the ones reported by Lass et al. [1], who reported the presence of both *T. gondii* DNA (real-time PCR and loop-mediated isothermal assays) as well oocysts (by using microscopy) in the air samples that were collected using gelatin filters. This may be due to different methods employed by our team. Future studies should focus on collecting soil samples from the fields in the areas with the highest seroprevalence for *T. gondii*, as well as utilizing the methods by Lass et al. [1] to collect air-borne dust samples to see if the results reported by them can be replicated.

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This work was supported by the U.S. Food and Drug Administration through the cooperative agreement FDU.001418 (TTP). Additional funding for this study was provided by the Mid-Atlantic Nutrition Obesity Research Center (NORC) Pilot & Feasibility Project (Postolache, PI),

a sub-award of the parent grant P30DK072488 (Simeon I. Taylor, Program Director).

Pteridines - Official journal of International Society of Pteridinology (ISP)

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Pteridines was established in 1989 as the official journal of the International Society of Pteridinology. In 2013 publishing duties was taken over by De Gruyter, and since the beginning of 2018, the journal was switched from conventional to Open Access publication model. After this change, the repository of all issues of Pteridines is available for everybody, without any limitations. The new model is also connected with shifting the costs of the publication process from the viewers to the authors - article processing charges are covered by them or on behalf of them. In return, authors receive numerous benefits e.g. free language assistance or potentially increased citation. The first year of Open Access in Pteridines could be summarized by growth in website traffic. In 2018, the views of the website increased by about 4.4 times, compared to 2017, which led to a higher number of articles downloaded (2.7 times more than in 2017). Besides the promising statistics, it is necessary to consider, how the Pteridines journal can be further developed. It appears that one of the key actions which should be taken is strengthening cooperation between the Society and the Publisher. A higher number of submissions from the Society, more citations, and dissemination of the information about the journal could increase the appeal of the journal in the scientific world and improve its rankings. Other activities also should be continued - marketing at the journal and article level as well as applying to appropriate scientific bases (such as PubMed) should aim to bring the submissions from scientists outside of the Society, which allows the journal to operate smoothly.

We express the hope, that these actions will consolidate the position of Pteridines and make it a source of pride for the International Society of Pteridinology and De Gruyter Publishing House.

Biosynthesis of riboflavin and pantothenic acid is crucial for virulence of *Aspergillus fumigatus*

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Aspergillus fumigatus is the most prevalent airborne fungal pathogen causing invasive fungal infections in immunosuppressed individuals. Limitations in antifungal therapy arise from non-specific symptoms of infection, poor diagnostics and comparatively few options for treatment. The aim of this study is to explore the metabolism of *A. fumigatus* on a comprehensive scale as essential virulence determinant to generate a collection of *A. fumigatus* strains with a focus on primary metabolism to target fungal pathways that are absent in mammals. Based on the annotated genome of *A. fumigatus*, metabolic network reconstruction served to identify fungal-specific pathways and key reactions. Predictions for unique enzymes resulted in a candidate list of genes, the inactivation of which is likely to result in an auxotrophic phenotype. The virulence potential of the generated auxotrophic mutant strains was then analyzed in various host niches. We identified two *A. fumigatus* pathways that are essential for growth in minimal medium: biosynthesis of the vitamins riboflavin and pantothenic acid. Inactivation of biosynthesis of both vitamin biosynthetic pathways resulted in attenuated virulence of *A. fumigatus* in murine models for invasive aspergillosis with intranasal and systemic infection. These results characterized the availability of nutrients in the host niche and reveal targets for development of novel antifungal therapeutic approaches.

Fungal infection in cystic fibrosis: first steps towards an *in vitro* model

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Cystic fibrosis is the most common hereditary disease among Caucasians. A mean prevalence of 0.737 per 10,000 in the European Union and an annual incidence of 1 per 3,500 in Austria were reported in 2004. The fibrosis of the respiratory tract is driven by episodes of fungal infections and inflammatory reactions. Among the major fungal agents are *Aspergillus fumigatus, Scedosporium apiospermum, Exophiala dermatitidis* and *Candida albicans*. Knowledge on the impact and management of fungal infections and collonisations is limited.

There is a substantial lack of information on cellular events that may favor and/or be provoked by fungal infections. To explore host fungi interactions in more detail, we aimed to mimic the fungal – epithelial cell interaction and infection in *in vitro* model systems.

The lung epithelial cell lines A549 and Calu-3 were applied, as these are frequently used respiratory models in drug research. Cells were grown as air-liquid interface cultures and co-incubated with the fungi mentioned above. So far, microscopy analysis revealed that fungi did not penetrate cell layers with intact barriers but grew and formed hyphae on the mucosal layer. However, if the epithelial barrier was injured, invading fungi could be observed though the penetration of the cell layer remained sparse. Being interested in signaling changes, we investigated the regulation of immunologically relevant pathways. Though results are still preliminary, we found distinct patterns of pathway activity in the co-presence fungi. Further investigations on the expression of target genes are necessary to get a better insight in the activated signaling cascades. In addition, the application of different cell models is expected to further enhance our understanding of fungal-host interactions in cystic fibrosis setting.

Indoleamine 2,3-dioxygenase-1 signalling response of the patients with chronic calculous cholecystitis

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Majority of gallstones are found to be colonized with a bacterial biofilm, even though the bile culture is negative. Biliary epithelial cells express Toll-like receptor-4 and form a receptor complex which mediate antigen-pattern recognition and promotes Type-1 interferon (IFN) production. Type-1 IFN production may suppress innate and adaptive immunity by inducing indoleamine 2,3-dioxygenase (IDO1), which promotes the immune tolerance via tryptophan (Trp)/kynurenine (Kyn) pathway. Aim of this study was to evaluate whether the IDO1 activity support the immune tolerance and continuation of inflammatory process in the chronic calculous cholecystitis (CCC) patients. In this study both CCC and their matched gallstone-free control groups were consisted of 26 patients. Bile samples were evaluated microbiologically. Serum total antioxidant capacity, nitrite plus nitrate concentrations, homocysteine, neopterin, Trp and Kyn levels as well as urinary biopterin and creatinine were measured. In addition, 8-hydroxydeoxyguanosine (8-OHdG) level, a biomarker of oxidative DNA damage, was investigated. Serum IDO1 activity was calculated by assessing the correlation between serum neopterin concentrations and the Kyn to Trp ratio. Out of 26 bile samples, 53.8% were shown to have bacterial growth. CCC patients showed a highly significant rise in Kyn, homocysteine and 8-OHdG concentrations, as well as Kyn/Trp ratios compared to their matched gallstone-free controls. The increase in IDO1 activities of CCC patients were confirmed with the significant correlation between the Kyn/Tryp ratio and neopterin levels. Even the bacterial growth could not be confirmed in all CCC patients, increased IDO1 activity in comparison to the gallstone-free patients was interpreted as the continuation of enhanced immune tolerance to bacterial load of gallbladder in CCC patients. Furthermore, increased IDO1 activity and oxidative DNA damage is common in chronic cholecystitis, suggesting a possible link between gallstone-related chronic inflammation and gallbladder carcinogenesis.

This study was supported by The Scientific & Technological Research Council of Turkey, Project No: 106S116.

Effects of dietary and genetic iron overload on mitochondrial function and consecutive metabolic pathways

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Iron is an essential co-factor for many metabolic processes, and mitochondria are the main sites of iron utilization as they need iron for mitochondrial respiration [1, 2]. Iron deficiency negatively affects cellular energy metabolism and iron overload increases the production of reactive oxygen species (ROS) through the Fenton reaction [2]. Therefore, the consequences of dietary and genetic iron overload on metabolic circuits and mitochondrial function were investigated in wildtype and $Hfe^{-/-}$ mice.

10 weeks-old male C57/BL6N wildtype mice and $Hfe^{-/-}$ mice were fed either with a low iron (>10 mg iron/kg) or a high iron diet (25 g iron/kg) for two weeks. Mitochondrial respiration was measured in liver homogenate using High-Resolution Respirometry (Oroboros Instruments, Innsbruck). Moreover, different molecular biological methods were applied for the determination of the tissue iron content, protein and mitochondrial gene expression.

Mitochondrial respiration in liver homogenate revealed no differences between the genotypes as well as the different diets. Tissue iron distribution was different according to genotype and diet exhibiting higher amounts of iron in mice fed the high iron diet and the $Hfe^{-/-}$ genotype seen especially in duodenum, liver and spleen. Furthermore, the expression of proteins involved in iron and mitochondrial metabolism in the liver confirmed these differences, as ferritin expression was increased in mice with iron overload. Moreover, the low iron diet led to an elevated expression of the transferrin receptor in the wildtype mice compared to the expression in the $Hfe^{-/-}$ knockout mice fed a low iron diet which was the same as in the mice fed a high iron diet. However, the expression of the marker protein for mitochondrial biogenesis, PGC1α, was the same in all groups. Mitochondrial gene expression exhibited differences between iron loading and iron deficiency that was independent of genotypes. In iron overload mitochondrial ferritin and mitoferrin 2 gene expression was increased whereas the expression of the PGC1α and the mitofusin 2 genes was unaltered.

The results showed that a knockout of the hemochromatosis gene and a diet rich in iron altered mitochondrial iron metabolism and function [2]. This might be an explanation for the fatigue that is a common symptom in iron overload and iron deficiency patients.

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Opening remarks / What is all about: Neopterin-biopterin-tryptophan in biomedicine

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The workshop series was initiated in the year 1982, when profs. Hans-Christian Curtius from Zurich, Switzerland, Wolfgang Pfleiderer from Constance, Germany and Helmut Wachter from Innsbruck, Austria, decided to meet annually for scientific exchange. As a location St.Christoph in the Arlberg region was chosen because it was almost right in the middle of the three university cities and because the workshop should allow not only scientific exchange but also provide a relaxing atmosphere with sports and skiing possibilities. All three researcher groups were scientifically active in the field of pteridines, a rather neglected topic at this time period. A few years later John A. Blair from Birmingham, UK joined the organizers due to his work on the role of pteridines in (inherited) metabolic disorders. Only a few years earlier the exiting findings on the role of 5,6,7,8-tetrahydrobiopterin in the enzymatic hydroxylation of aromatic amino acids were published by Seymour Kaufman (Bethesda, Maryland), [1]. In 1979, pteridine derivatives came in the focus of immunology when neopterin was reported to be released by human monocyte-derived macrophages upon stimulation with cytokines, the major stimulus being interferon-γ (IFN γ). The monitoring of neopterin concentrations turned out as a very sensitive and reliable biomarker of the cellular immune response [2], e.g., to detect allograft rejection and virus infections at an early stage, or to monitor of autoimmune pathologies. As a consequence, neopterin measurements became obligatory in Austria for the screening of blood donations to reduce the risk of pathogen transmissions. Thus, in addition to chemistry, clinical biochemistry, laboratory analytics and immunology became central topics of the workshops series. Lateron, the immunobiological link between activation of indoleamine 2,3-dioxygenase and neopterin formation became evident and provided better understanding of the development of neuropsychiatric symptoms like depression and dementia in patients suffering from immunopathologic conditions, e.g., why patients with cancer have an increased risk of depression on a molecular basis.

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Dissecting the AhR-IDO1 axis function in dendritic cell immunotherapy

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Dendritic cells (DCs) play a vital role in host immunity by inducing innate inflammatory responses to pathogens, efficiently priming naïve lymphocytes, reactivating memory T cells, and by promoting B cell activation. However, DCs are also important in maintaining immune homeostasis and self-tolerance. DC lineage development and the extent of DC maturation or activation can profoundly affect the capability of DCs to induce immunity and tolerance. Under steady-state conditions, DCs consist of three major types, based on developmental origin, surface markers, and functions: pDC, DC1 and DC2. Although DC subsets maybe programmed to direct either tolerance or immunity, appropriate environmental stimulation could result in complete flexibility of a basic program, inducing either immune activation or tolerance, depending on environmental conditions. One mechanism by which DCs may regulate tolerance involves the enzyme indoleamine 2,3-dioxygenase 1 (IDO1) a tryptophan (Trp) metabolizing enzyme, leading to Trp starvation, that exerts potent immunoregulatory effects when expressed in DCs. In this study, we analyzed the ability of L-Kyn to induce tolerogenic IDO1 pathway in different DCs subsets *in vitro* and in experimental tumor models *in vivo*. We show that inflammatory stimuli, like LPS, were able to induce IDO1 only in DC1, but not in DC2 or pDC, when DCs were treated as isolated cultures. In contrast, when LPS was added to cultures containing all three DC subsets, LPS could also induce IDO1 expression in DC2, which acquired tolerogenic function. Induction of IDO1 in DC2 involved a novel DC1-DC2 communication pathway mediated by a Kyn-AhR-RelB axis. Kynurenine produced by DC1 activates AhR in DC2 inducing IDO1 in a RelB-dependent manner. Importantly selective AhR or IDO1 deletion from cDC1 using DC1 specific Cre deletors led to rejection of a progressively growing tumor. These data suggest that tryptophan metabolites, produced by cDC1 cells can convert immunogenic DCs into tolerogenic DC subsets. These different functions amongst DCs may offer great potential to tune immune responses, harnessing subset-specific functions of DCs in the clinical setting.

Comparison of competitive ELISA and HPLC determinations of tryptophan in human serum

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Within the past decades the process of T1-type immune activation was observed to correlate with tryptophan (Trp) degradation. Accordingly low serum tryptophan concentrations are often found in patients suffering from clinical conditions, which go along with immune activation such as chronic infections or cancer and were demonstrated to be closely associated with low mood and disturbed quality of life [1]. In patients with malignant melanoma, decreased Trp concentrations were observed to be predictive for their survival [2].

Under conditions of immune activation and inflammation, Trp degradation is mainly stimulated by the pro-inflammatory cytokine interferon- γ (IFN-γ) resulting in the activation of the enzyme indoleamine 2,3-dioxygenase-1 (IDO), which converts Trp to kynurenine (Kyn). Thereby the Kyn-to-Trp ratio (Kyn/Trp) serves as an estimate for IDO activity [3] and correlates with various immune activation biomarkers like neopterin [4]. Importantly, Trp is the precursor molecule of serotonin synthesis, and the concentrations of Trp also can be used as a surrogate marker of serotonin availability in the brain. Although the transport of Trp into the brain via the leucine-preferring L1 system is in competition with the so-called large neutral amino acids (LNAA) and the ratio of Trp to LNAA determines the flux of Trp into the brain, the sometimes drastic lowering of serum Trp during immune activation can hamper serotonin biosynthesis [1] and the measurement of Trp outside the brain can provide some clue about the intrathecal situation.

Serum Trp measurements are usually performed by liquid chromatography (LC) methods but within the last few

years an increasing number of ELISA-based Trp methods have been developed. Aim of this study was to compare the results of determinations of serum Trp in sera of healthy blood donors by a competitive ELISA (Immundiagnostik, Karlsruhe, Germany) with a reversed-phase high performance LC (RP-HPLC) method [4]. Trp measurements in 80 serum samples obtained from patients with HIV-1 infection [5] revealed a mean value of 59.0 µmol/L (SD ± 11.7 µmol/L) which was slightly higher compared to ELISA results (53.3 µmol/L, SD ± 15.4 µmol/L). The correlation of the results from HPLC and ELISA was strongly positive ($rs = 0.731$, $p < 0.001$), and there was a significant inverse relationship between Trp concentrations measured by HPLC and neopterin concentrations by ELISA (BRAHMS, Hennigsdorf, Germany) ($rs = -0.579$, $p < 0.001$), which however was substantially weaker when the Trp ELISA results and neopterin concentrations were compared ($rs = -0.254$, $p < 0.01$).

We conclude, the evaluated ELISA approach can be recommended as a suitable complementary method, when no LC equipment is available. It represents a cost-effective and reliable method for studying Trp concentrations in blood serum samples.

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A method for the quantitative determination of tryptophan metabolites in serum by LC-MS/MS: validation and its application - a preliminary study

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The aromatic amino acid tryptophan is the least available of all proteinogenic amino acids. As such, tryptophan availability is an important factor in the regulation of protein biosynthesis. This is one important reason for the immune system to utilize tryptophan starvation to restrict unwanted proliferation of pathogens and malignant cells. The activation of the tryptophan-degrading enzyme IDO-1 is triggered mainly by interferon (IFN)-γ, likewise the formation of the cellular immune activation marker neopterin. IDO-1 catalyzes the first and rate-limiting step of tryptophan breakdown to kynurenine, and the kynurenine-to-tryptophan ratio (Kyn/Trp) was proposed as an estimate of IDO enzyme activity in the early 1990s [1]. Since then, a variety of new biological functions of tryptophan downstream metabolites has been discovered. We developed and validated a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantification of tryptophan, kynurenine, 3-hydroxyanthranilic acid, anthranilic acid, kynurenic acid, quinolinic acid, picolinic acid and neopterin in human serum and plasma. Serum samples were spiked with isotopically labeled internal standards and sample preparation consisted of generic protein precipitation. The analytes eluted from a monolithic reverse phase C18 column during chromatographic runs and were analyzed by MS/MS in positive ESI mode. We applied the method to a sample cohort of end-stage cancer patients and healthy controls. Significantly elevated Kyn/Trp ratios were confirmed in samples from patients.

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Immune activation in older-aged individuals is associated with genetic instability *(or vice versa)*

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Older adults are confronted with an increased risk of disability and mortality, a condition which is circumscribed as frailty. Since the aging process is associated with immunosenescence involving both increased inflammation and tissue damage, while capacities of defense mechanisms decline, and recently an association between frailty and genomic instability has been reported in individuals aged 65 years and over, the question arose whether genomic instability might be associated with a status of chronic immune activation that develops with older age or vice versa.

In this study, we analyzed the potential association between the micronucleus frequency in lymphocytes and buccal cells as indicators of genetic instability, as well as concentrations of serum soluble biomarkers of immune activation like soluble 75 kD tumor necrosis factor-a receptor (sTNF-R75), macrophage marker neopterin and tryptophan breakdown as is estimated by the kynurenine to tryptophan ratio (Kyn/Trp) in 239 individuals (79 females) older than 63 years of age (range: 64-102).

Higher frequencies of binucleated buccal cells were associated with higher sTNF-R75, Kyn/Trp, neopterin and lower nitrite concentrations (all $p < 0.001$). Less strong but still highly significant correlations were observed also for micronuclei in lymphocytes ($p < 0.001$) and gamma-H2AX ($p < 0.05$), a biomarker for DNA double-strand breaks, and in the opposite direction for pyknotic buccal cells (all $p < 0.05$). No significant relationship existed for the other parameters, e.g., micronuclei, cells with condensed chromatin, karyorrhectic cells and karyolytic cells (all parameters evaluated in exfoliated buccal samples), as well as the %-repair capacity, with any of the soluble biomarkers.

Our results reveal a close relationship between concentrations of biomarkers of immune activation, especially for sTNF-R75, Kyn/Trp and neopterin, and genetic instability in older adults. Data imply a role of the macrophages activated by Th1-cytokine interferon-γ, which strongly induces not only neopterin production and tryptophan breakdown via indoleamine 2,3-dioxygenase-1 (IDO-1), but also the formation of reactive oxygen species (ROS), which may promote genetic instability due to oxidative stress development.

This work was supported by Xunta de Galicia (ED431B 2016/013, ED431C 2017/49, and FrailNet network IN607C 2016/08). VV was supported by a Xunta de Galicia postdoctoral fellowship (ED481B 2016/190-0), LLL by the "Ramon y Cajal" Postdoctoral Senior Grant (RYC-2015-18394) from the Spanish Ministry of Economy, Industry, and Competitiveness, co-financed by the European Social Fund, and by the Xunta de Galicia (project ED431F 2017/09), and DM-P and MS-F Sánchez-Flores by INDITEX-UDC fellowships.

Tryptophan, kynurenine and cardiovascular parameters in individuals with mental disorders

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Tryptophan is an essential amino acid and the precursor to the neurotransmitter serotonin. Beside other neurotransmitters, serotonin plays a major role in the pathogenesis of depressive episodes and anxiety [1-3]. Further, serotonin also has many others functions centrally and peripherally in the human body. In chronic stress, especially in depression, alterations in tryptophan metabolism are known. Under these circumstances, pro-inflammatory conditions are leading to tryptophan depletion, central serotonin deficiency and increased activity of the kynurenine pathway [3]. By measuring the heart-rate variability (HRV), as the non-invasive evaluation of the beat-to-beat heart rate dynamics, the psychosomatic influences of stress on the autonomous nervous system function can be examined [4]. For instance, a higher stress induced sympathetic tone is leading to a reduced HRV and promotes inflammatory processes. Therefore, high stress-levels might be associated with both impaired HRV and increased tryptophan breakdown. In this report we present the correlation between current psychiatric symptoms, tryptophan breakdown and altered HRV in a sample of psychiatric patients during a six-week psychiatric rehabilitation programme. Possible associations could underline the role of tryptophan metabolism as a common denominator between mental illness and cardiovascular diseases.

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Imaging – inn between life and death

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Herein we present a short 10 year retrospective view on Real Time Live Confocal Imaging in Innsbruck. In a synergistic approach several cellular questions in between life and death were addressed.

We applied Real Time Live Confocal Microscopy using two spinning disc systems (Perkin Elmer/Zeiss/Olympus). One method called Biopsychronology which uses unfixed biopsies for the analysis of cell viability and vitality (suitable for liquid as well as solid biopsies) will be described in more detail [1].

Thanks to the collaboration with different Departments in Innsbruck as well as abroad, we could show that Real Time Live confocal Imaging is applicable in a wide variety of applications. Liquid as well as solid biopsies were used to analyse cell viability and vitality [1, 2]. Two or three dimensional *in vitro* cell culture models [3], static as well as dynamic can also be combined with Real time live confocal imaging, thereby allowing novel insights in real time without the need of fixation. Even molecules and the activity of coagulation factors may be visualised [4].

As shown with Biopsychronology such novel insights can also be transferred from bench to bedside.

Herein we convincingly show the power of an imaging modality which is well suited to be used as a visualization tool in a wide range of projects dealing with key cells and molecules in between life and death.

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In vitro immunomodulatory properties of phytochemicals

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A broad range of natural products are known to interfere with inflammatory and oxidative processes. In inflammation, the redox sensitive tryptophan metabolism plays a key role in several disorders that are associated with chronic immune activation ranging from infection, malignancies, neurodegenerative to cardiovascular disorders [1]. This pathway has emerged as a preferred target for immunomodulation by a variety of substances including drugs, phytochemicals and nutritional components [2]. The antioxidant flavonoid kaempferol is nearly ubiquitously distributed in edible and medicinal plants and has previously shown to exert a broad range of pharmacological activities.

In this study we demonstrated suppressive effects of kaempferol on indoleamine 2,3-dioxygenase (IDO-1) activity the model system of mitogen stimulated human peripheral blood mononuclear and THP-1 cells [3]. Moreover, *in silico* docking studies revealed a potential interaction of kaempferol with the catalytic domain of IDO-1. Other interferon-γ dependent signaling cascades were attenuated in parallel, such as the formation of the oxidative stress marker neopterin. In contrast to these anti-inflammatory activities, kaempferol superinduced NF-κB signaling in LPS-treated THP-1 myelomonocytes, thereby increasing the expression of interleukin (IL) 1B, IL-6 and tumor necrosis factor alpha.

Natural compounds like kaempferol are able to interfere with immunoregulatory pathways by targeting different immune cell types. Data underline the advantage of integrating the testing parameters IDO-1 and GTP-CH-I activity in the search for novel anti-inflammatory compounds. As both neopterin and Kyn/Trp are biomarkers, which have been used for the monitoring of several human diseases, there is high relevance of such parameters for the *in vivo* situation [4].

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Dysbiosis in patients with chronic diseases: probiotics as possible treatment option?

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The intestinal microbiome has been shown to play a substantial role in many physiological functions of the host organism. An altered composition of the gut microflora, commonly referred to as dysbiosis, may therefore contribute to the development of a broad range of systemic diseases. The benefit of probiotics as a potential treatment option in chronic diseases associated with dysbiosis is currently being investigated in many clinical studies and meta-analyses.

In patients with antibiotic-associated diarrhea, Clostridium difficile-associated diarrhea and as a concomitant treatment in the eradication therapy for H. pylori a positive effect of probiotic treatment was seen [1-3]. Concerning inflammatory bowel disease and irritable bowel syndrome there are controversial results [4-6]. Probiotics were also shown to lower blood pressure in hypertensive patients [7] reduce fasting plasma glucose and HbA1c in patients with type 2 diabetes [8] and improve the lipid profile in those patients [9]. Meta-analyses also showed reduced episodes of upper respiratory tract infections under probiotic therapy [10, 11].

In patients with rheumatoid arthritis the disease activity score was improved, while inflammatory parameters did not change [12]. A single trial indicated improvement of cognitive parameters in patients with Alzheimer's disease [13] and several studies provide evidence of beneficial effects of probiotics on depressive symptoms [14–16].

To conclude, probiotics might have beneficial effects in patients with chronic diseases. Further research is required to investigate the distinct effects of specific probiotic microorganisms and to determine the effects of different treatment durations.

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Iron supplementation leads to an increased bacterial load independent of the basal iron status

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Iron deficiency is a major health concern specifically in children, which can result in impaired mental development and growth retardation. However, iron supplementation programs to children in low-income countries resulted in an increased morbidity and mortality from infections. Iron is an essential nutrient for many microbes and has subtle effects on immune function. Thus far, limited information was available on the possible underlying mechanisms of comprehensive iron supplementation followed by bacterial infections.

We assessed this question in mice that we fed an iron deficient and iron adequate diet for four weeks. Half of the mice received an oral iron supplementation for three days and the other half stayed on their initial diet. Following this, the mice received an intraperitoneal infection with *Salmonella typhimurium*, an established model for sepsis. Control mice received intraperitoneal PBS.

Iron supplementation leads to a higher number of colony forming bacterial in the spleen and liver

independent of the basal iron status. This observation was paralleled by an increase of the pro-inflammatory cytokine IL-6, the master iron regulator hormone hepcidin and a reduction of the only known iron exporter ferroportin.

Our results demonstrate that an iron supplementation independent of the basal iron status followed by an infection with an intracellular bacteria like *Salmonella* can lead to a more severe bacterial infection. We anticipate that knowledge about our findings can lead to more careful iron supplementation even in iron deficient children.

How acute and chronic physical disease may influence mood – an analysis of neurotransmitter precursor monoamine levels

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Patients with somatic diseases are more likely to develop depression than physically healthy individuals, and comorbid depression has been shown to incrementally worsen patients´ health. Physical conditions are known to influence neurotransmitter precursor amino acids, changes in which are associated with depressive symptoms. In this prospective study we investigated neurotransmitter precursor amino acids levels in patients with acute and chronic physical disease and evaluated their association with depressive symptoms [1].

177 subjects with and without chronic medical comorbidity (factor: chronic physical disease) admitted to the trauma and orthopaedic surgery ward for a surgical intervention (factor: acute physical disease) were included in the analysis. Chronic medical comorbidity was scored using Charlson Index and depressive and anxiety symptoms using the Hospital Anxiety and Depression Scale (HADS, factor: mental health). The effect of covariates was also evaluated. C-reactive protein (CRP), neopterin, kynurenine/tryptophan (Kyn/Trp) and phenylalanine/tyrosine (Phe/Tyr) were analysed by HPLC or ELISA prior to surgery and at discharge. Mixed Model as well as correlation analyses were performed.

CRP and neopterin levels were influenced by the factors "acute physical disease" (both $p < 0.001$) and "chronic physical disease" ($p = 0.024$, $p = 0.001$ respectively). Phe/Tyr, an index of the catecholamine pathway) was related to the factors "acute physical disease" ($p < 0.001$) and "mental health-depression" ($p = 0.012$), while Kyn/Trp (an index of the kynurenine pathway affecting also serotonin) was associated with "chronic physical disease" ($p = 0.005$). No significant effect of "mental health-anxiety" was found. The effect of "mental health-depression" on Phe/Tyr was more pronounced in females (gender $p = 0.003$). Differences in HADS depression values correlated with changes in Phe/Tyr and both correlated with CRP values.

In conclusion, inflammatory reactions related to acute or chronic physical conditions can influence the availability of neurotransmitter precursor amino acid levels and these changes are associated with mental health.

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Impact of mitogens and oncogenes on p27 tyrosine phosphorylation and cell cycle control

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The cell cycle inhibitor $p27^{Kip1}$ controls cell cycle progression by binding to and inhibiting the activity of cyclin-CDK complexes. Low nuclear p27 levels are detected in proliferating cells and have been associated with tumor development. Phosphorylation strongly influences p27 function and stability. Thereby, tyrosine phosphorylation at residue 88 leads to partial activation of p27 bound cyclin-CDK complexes and can initiate the SCF-Skp2 ubiquitin-dependent degradation of p27.
Several tyrosine kinases of the non-receptor- and receptor tyrosine kinase family including Src, Lyn, Abl, JAK2 and FLT3 can phosphorylate p27 on residue 88. Stimulation with mitogens that lead to the activation of the identified kinases induce p27 tyrosine-88 phosphorylation at the endogenous level, suggesting that this mechanism can be employed to restore partial cyclin D-CDK4 activity to enable G1 phase progression in response to diverse

mitogenic stimuli. Beside the presence of tyrosine-88 phosphorylated p27 in cancer cell lines expressing the oncogenic tyrosine kinases BCR-Abl, JAK2-V617F and FLT3-ITD, we were able to detect this p27 modification also in FLT3 wildtype and FLT3-ITD expressing primary blast cells from acute myeloid leukemia patients. Treatment of these leukemic cells by specific tyrosine kinase inhibitors reduced tyrosine-88 phosphorylation of p27 suggesting that the thereby restored p27 function may inhibit cell proliferation and contribute to successful drug treatment.
Supported by the Austrian Science Foundation (P24031) and the Austrian Cancer Society/Tirol (16005)

Phyllobilins as bioactive constituents of medicinal plants - investigations on immunomodulatory activities

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The focus of our studies is on a family of plant derived natural products, the phyllobilins. These tetrapyrrolic compounds arise from the degradation of chlorophyll and are therefore ubiquitous in nature. Phyllobilins have been discovered roughly 25 years ago and have since then been detected in leaves, the peels of ripening fruit, and vegetables [1,2]. Despite their abundance and the fact that they are part of human nutrition, phyllobilins are surprisingly unexplored in regard to their physiological properties [3]. The main phyllobilins are colorless and can be oxidized to yellow chlorophyll catabolites (phylloxanthobilins) that were also shown to occur naturally [4]. In this study, we focus on phylloxanthobilins in medicinal plants and their possible contribution to the pharmacological effects of the marketed medicinal products of the plant. In earlier experiments, it was shown that phylloxanthobilins possess interesting bioactivities, such as antioxidant activity, and - using the PBMC model - a phylloxanthobilin was shown to have anti-inflammatory properties (unpublished). Our preliminary data indicate that phylloxanthobilins occurring in medicinal plants play a role in the pharmacological effects attributed to extracts or medicinal preparations and should be considered when studying the bioactivities of this plant.

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Immune status, depression and quality of life in patients with recurrent infections

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Patients with recurrent infections have to be evaluated for possible reasons underlying their increased susceptibility for infections. A physical examination has to be performed to exclude anatomic obstructions and primary or secondary causes of immunodeficiency, like chronic diseases (HIV, Diabetes, Malignancy, etc.) or current treatment favoring recurrent infection (Radiation therapy, Immunosuppressive agents, etc.)

We are currently investigating the immune status of patients with recurrent infection, their mood and quality of life by questionnaires (BDI-II, FSS, SF36). Furthermore we test whether immune-mediated changes of tryptophan and phenylalanine metabolism are related with neuropsychiatric and digestive alterations in patients. The main aim of our double blind, placebo controlled study is to investigate, whether treatment with a multistrain probiotic (OMNi-BiOTiC® STRESS Repair, Allergosan, Graz; for 6 months) is effective to improve patients´ symptoms.

Until now we have 33 patients participating in our study (24 female, 9 male, mean age 41 years). More than 70% of the patients had at least 5 infections in the last year. 49% had respiratory symptoms, followed by GIT, Urinary Tract and skin symptoms. 18 patients were depressed and 24 patients reported about mild to severe digestive disorders at baseline.

These preliminary data indicate that recurrent infection does not only impair the physical, but also the mental health of patients.

Tryptophan and phenylalanine metabolism and depression in patients with solid tumors

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Depression and impaired quality of life (QoL) are very common complaints in cancer patients. There is increasing evidence that immune-mediated changes in tryptophan and phenylalanine metabolism could be involved in the development of mood changes.

During the immune response to malignant tumors the th1-type cytokine IFN-γ activates the enzyme IDO to degrade tryptophan via the kynurenine pathway. IFN-γ release also enhances the oxidation of tetrahydrobiopterin (BH4), the essential cofactor for the formation of tyrosine from phenylalanine leading to reduced concentrations of the catecholamine precursor tyrosine. 152 patients with solid tumors completed the questionnaires EORTC QLQ-C30 and BDI-II to measure QoL and depression. Immune activation parameters as well as parameters of tryptophan and phenylalanine metabolism were determined in the patients' sera.

Deteriorated QoL was associated with increased depressive symptoms. 40.1% of the study participants and 47.4% of the anemic patients were suffering from depression.

Elevated neopterin concentrations correlated with enhanced tryptophan degradation and a disturbed phenylalanine metabolism. Impaired QoL was associated with tryptophan catabolism and reduced hemoglobin concentrations. Female patients with deteriorated QoL also showed changes in the phenylalanine metabolism. Men with depression showed lower tryptophan concentrations.

The results of our study suggest that immune-mediated disturbances of tryptophan and phenylalanine metabolism as well as cancer-related anemia might be involved in the development of depression and might also contribute to a deteriorated quality of life of patients.

Depression and quality of life in patients with post-infectious fatigue

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After infections (especially with viruses) a significant percentage of patients suffers from post-infectious fatigue. Currently no evidence-based treatment is available. Gastrointestinal dysfunction and alterations of the gut microbiome have been reported in patients with chronic fatigue syndrome and in patients with depression, and probiotics appear to be beneficial in these patients.

In our prospective, double blind, placebo controlled pilot study, we investigate the quality of life, depression, fatigue and immune status in patients with post-infectious fatigue (by questionnaires Fatigue Severity Scale (FSS), Beck's Depression Inventory II (BDI-II) and Short Form 36 (SF-36)). Furthermore we also determine immune-mediated alterations of tryptophan and phenylalanine metabolism and their relationship with neuro-psychiatric symptoms and gastrointestinal dysfunction. The aim of our study is to test whether treatment with a multistrain probiotic is able to improve patients´ fatigue, mood and digestion. 70 participants are randomized to receive either 7.5 billion colony forming units of different strains of Lactobacilli and Bifidobacteria or placebo for 6 months.

Until now, 33 patients have been included. At baseline, 17 patients suffered from depression, 75 % of them had severe fatigue (mean FSS 5.39) and reported about a significantly impaired role function due to physical and emotional problems. Interestingly, laboratory parameters were mostly within reference ranges in our patients.

Patients with post-infectious fatigue often suffer from depression and impairment of their physical and emotional healthiness. We try to evaluate if probiotics might be a treatment option for these patients.

LC-MS/MS analysis for the structural characterisation of ester and ether lipids in biological samples

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Every living cell is structured by biological membranes which include the plasma membrane as well as cellular organelles, such as for example the endoplasmic reticulum, peroxisomes, and mitochondria. A well-defined lipid mixture enables the necessary selective permeability. In mitochondrial membranes the lipid class of cardiolipins (CL) is of special importance. CLs are dimeric phospholipids exclusive for mitochondria, which are involved in many mitochondrial core functions such as the stabilisation of respiratory complexes, cristae formation, and apoptotic signalling. As known and confirmed by our measurements we find different molecular CL species distributions in various tissues. Especially heart is of interest, because there the tetralinoylcardiolipin accounts for about 80 % of total cardiolipins while in brain tissues we found a broad distribution of CLs with longer and more unsaturated fatty acyl residues compared to all other tissues. To elucidate which factors determine the tissue specificity of CLs we choose to look at connected phospholipid pools which can transfer specificity through a remodelling process. Therefore, we quantified all major phospholipid pools and performed a Fisher distance analysis. Interestingly, while in many tissues the phosphatidylcholines can partly explain the measured CL profiles, in brain ether-bound phospholipids were found to be the structurally most similar lipid class. However, in heart no similar lipid pool was found in this analysis. In summary phospholipid remodelling can help to explain the CL profile in some tissues while others show a remodelling independent behaviour.

On vitamin B_{12}, antivitamins B_{12} and (a little about) folate

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Vitamin B_{12} is an essential micro-nutrient for us humans, whose physiological effects are still incompletely understood [1]. B_{12}-deficiency is a wide spread condition, which also interferes with folate metabolism and its functional deficiency [1] (the 'methyl-tetrahydrofolate trap'). In this lecture synthetic approaches to novel analogues of the organometallic B_{12}-cofactors are presented, which have been designed to explore hidden facets of B_{12}-biology in humans and animals, concentrating on two exceptional, so called 'antivitamins B_{12}' [2]. These B_{12}-antimetabolites derive their potential roles from their structural mimicry of the natural B_{12}-cofactors and their strongly altered chemical reactivity and cofactor inactivity [2], inducing B_{12}-deficiency in animals [3]. Rhodium analogues of the cobalt containing natural B_{12}-cofactors [4] represent particularly well suited 'antivitamins B_{12}' [2], whose potential as enzyme inhibitors [4] and effective antibiotics [5] has been explored in pioneering studies. Hence, the biologically important structural properties of B_{12}-cofactors and of antivitamins B_{12} are discussed, as well as some of their vital interactions with B_{12}-binding proteins and B_{12}-dependent enzymes.

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Tryptophan and phenylalanine metabolism in patients with immunodeficiency due to immunoglobulin deficiency

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Patients with recurrent infections often have immunoglobulin deficiency and benefit from immunoglobulin treatment. We investigated the immune status of 53 patients (36 women, 17 men, mean age 53 years) with current immunoglobulin treatment by questionnaire and laboratory measurements. Furthermore we assessed immune-mediated changes of tryptophan and phenylalanine metabolism. Patients had 5.2 infections/year, most patients had respiratory infections (n=33). 12 patients had to be admitted to the hospital within the last year, many patients suffered from co-morbidities (55 %

respiratory diseases, 53 % cardiovascular disease, 23 % St. p. cancer disease). Interestingly, only 7 patients reported about fever during infections. Under immunoglobulin therapy, nearly all patients had immunoglobulin levels within the reference range. Tryptophan degradation was slightly enhanced and a higher Kyn/Trp was associated with higher inflammatory markers like neopterin, ESR, and procalcitonin.

Patients presented with increased phenylalanine and decreased tyrosine concentrations compared to healthy controls. Patients with more infections had higher tyrosine levels and a lower Phe/Tyr ratio, while immunoglobulin levels were not correlated with the number of infections. Patients treated with proton pump inhibitors (n=26) had lower tryptophan and haemoglobin levels.

Immune-mediated changes of tryptophan metabolism are only slightly enhanced in patients with immunoglobulin deficiency. However, phenylalanine-tyrosine metabolism alterations appear worthy being investigated by further studies.

Are immune activation and inflammaging involved in the physiopathology of frailty in older adults?

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Since chronological age is not a proper indicator of aging process, the concept of "frailty" has been recently proposed as a more precise measurement of aging signs and symptoms. Frailty is defined as a multidimensional geriatric syndrome characterized by a progressive physiologic decline in multiple body systems, manifested by loss of function and physiologic reserves, and increased vulnerability to disease and death. Age-related modifications in the immune system culminate in a chronic pro-inflammatory state, called "inflammaging". Physiopathological mechanisms of frailty are not clearly revealed yet, but it has been postulated that inflammaging may have a role in frailty development. On this basis, the main objective of the present work was to assess the possible role association of chronic low-grade immune stimulation on and frailty status in the elderly. To that aim, a cross-sectional study was conducted in a population of Spanish older adults (aged 65 years and over) classified according to their frailty status following the Fried's phenotype criteria [1]. Concentrations of neopterin, tryptophan and phenylalanine metabolism parameters, nitrite, and a set of inflammatory mediators were determined in plasma samples, and their relationship with frailty status were analyzed. Results obtained showed significantly higher concentrations of neopterin, and of kynurenine/tryptophan and phenylalanine/tyrosine ratios, and significantly lower concentrations of tryptophan, nitrite and tyrosine in frail individuals as compared with non-frail subjects. All inflammatory mediators evaluated were also increased with frailty severity. Significant correlations observed between immune biomarkers indicate they change in parallel, thus pointing to interrelated causes. Present results are consistent with an additional degree of endogenous immune stimulation in frail older adults, and provide support to the involvement of inflammaging in frailty status.

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This work was supported by Xunta de Galicia (ED431B 2016/013, ED431C 2017/49, and FrailNet network IN607C 2016/08). VV was supported by a Xunta de Galicia postdoctoral fellowship (ED481B 2016/190-0), LLL by the "Ramon y Cajal" Postdoctoral Senior Grant (RYC-2015-18394) from the Spanish Ministry of Economy, Industry, and Competitiveness, co-financed by the European Social Fund, and by the Xunta de Galicia (project ED431F 2017/09), and DM-P and MS-F Sánchez-Flores by INDITEX-UDC fellowships.

Inflammation, iron and vitamin D metabolism in different cardiomyopathy etiologies

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Immune activation and disturbances in iron and vitamin D metabolism are all found in patients with cardiomyopathy. However, investigations about differences in immune activation or disturbances in iron and vitamin D metabolism between the different cardiomyopathy aetiologies are rare.

Parameters of iron metabolism (haemoglobin, iron, transferrin, transferrin saturation, ferritin, hepcidin) vitamin D metabolism (Ct-FGF23, parathormone, phosphate, vitamin D) and inflammation (C-reactive protein and neopterin) were determined in 149 patients (98 men, 51 women) with non-ischaemic cardiomyopathy.

Patients with amyloid cardiomyopathy showed a stronger association with TH1 immune activation reflected by neopterin and higher ferritin and hepcidin levels than other cardiomyopathy aetiologies. Interestingly, C-reactive protein but not neopterin levels were significantly higher in patients with inflammatory cardiomyopathy. When investigating for differences in iron and vitamin D metabolism, ferritin levels were significantly higher and Ct-FGF23 levels significantly lower in patients with virus positive compared to patients with virus negative inflammatory cardiomyopathy.

This study indicates that there are some differences in the grade of immune activation as well as in iron metabolism disorders between different cardiomyopathy aetiologies. Further studies with larger patient cohorts are needed to investigate these findings more precisely.

Knock on effect from periodontitis to the pathogenesis of Alzheimer's disease?

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Alzheimer's disease (AD) has inflammatory components which can be enhanced by systemic inflammation. There is growing evidence that chronic periodontitis drives systemic inflammation, consecutively neuroinflammation - a relevant pathomechanism in the course of neurodegeneration and finally AD. A link might exist between oral pathogens and AD. This is of special interest as in AD patients because there is an age-related incidence of chronic periodontitis.

In this pilot study, 20 consecutive patients with AD (aged 78.1 ± 2.2 y, 9 females), recruited between May and September 2018 were investigated; 35% of these patients with periodontitis were found to harbor one or more pathogenic periodontal bacteria. The presence of *Porphyromonas gingivalis*, the key pathogen and one of the species involved in chronic periodontitis, was found to be associated with lower mini mental state examination scores (13.4 + 3.68; U = 2.239, p <0.03) and with a tendency to lower scores in the clock drawing test (3.00 + 1.64; U = 1.989, p = 0.056). Further associations between lower serum neopterin concentrations and the presence of Treponema denticola (6.14 + 0.65 vs. 9.58 + 0.73 nmol/L; U = 2.533, p <0.01; Mann Whitney U-test) as well as lower serum kynurenine concentrations were found in AD patients positive vs. negative for Tannerella forsytia (1.64 + 0.17 vs. 2.16 + 0.20; U = 1.980, p <0.05).

Data indicates a correlation of specific periodontal pathogens with cognitive decline. Treponema denticola and Tannerella forsytia may down-regulate the host response in AD patients leading to a reduced inflammation. Albeit still preliminary, findings of this pilot study suggest an altered salivary microbiome to provide a causal link between chronic periodontitis and cognitive decline in AD.

Microbicidal activity of N-chlorotaurine in the presence of lung epithelial cells

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N-chlorotaurine (NCT) is an endogenous active chlorine compound that can be used as an antiseptic in different body regions. Recently, tolerability of inhaled NCT has been demonstrated in humans so that it is of interest for future treatment of cystic fibrosis. In the present study, we tested the bactericidal and fungicidal activity of NCT in a lung cell culture model.

Bacteria (Staphylococcus aureus, Pseudomonas aeruginosa) and fungi (Candida albicans, Exophiala sp.) were added to monolayers of lung epithelial cells, and after 4 h NCT was added. After different incubation times, aliquots from extracellular fluid as well as from intracellular one after lysis of the cells with NP-40 were removed and quantitative cultures were performed.

NCT at the therapeutically applied concentration of 1% (55 mM) killed the test pathogens within 15 - 30 min. Killing by 0.3% NCT lasted up to 4h dependent on the pathogen. 0.1% NCT was the threshold concentration for killing since this amount of oxidation capacity was consumed by reactions with the organic compounds of the medium within 3h.

In conclusion, NCT in therapeutic concentration demonstrated its microbicidal activity in the presence of lung epithelial cells.

Development of an LC-MS/MS assay for the quantitation of tryptophan and associated metabolites in plasma, feces, and brain tissue

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We developed a high-throughput ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) assay for the quantification of tryptophan, 5-hydroxytryptophan, kynurenine, kynurenic acid, 3-hydroxykynurenine, quinolinic acid, quinaldic acid, anthranilic acid, 3-hydroxyanthranilic acid, xanthurenic acid, picolinic acid, indole-3-propionic acid, 5-hydroxyindoleacetic acid, nicotinic acid, nicotinamide, serotonin, melatonin, dopamine, and neopterin in human EDTA plasma, human feces, and pig brain tissue. The assay is based on a 96-well plate format, requires 100 µL plasma or 25 mg feces/brain, respectively, and has a run time of 5.5 min per sample. The sample extraction workflow was optimized for the three tested matrices. The assay enables the assessment of concentrations of the listed analytes in the respective biological matrices rapidly and reliably.

Differences in tryptophan metabolism between euthymic, hypomanic and depressive episodes

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The kynurenine pathway is involved in inflammatory diseases. Alterations of this pathway have been shown in psychiatric entities as well. The aim of our study was to determine whether specific changes in kynurenine metabolism are associated with current mood symptoms in bipolar disorder.

Sum scores of the Hamilton Depression Scale (HAMD), Beck Depression Inventory (BDI-II) and Young Mania Rating Scale (YMRS) were collected from 156 bipolar individuals to build groups of depressive, manic and euthymic subjects according to predefined cut-off scores. Severity of current mood symptoms was correlated with the activities of the enzymes kynurenine 3-monooxygenase (KMO, ratio of 3-hydroxykynurenine/ kynurenine), kynurenine aminotransferase (KAT, ratio of kynurenic acid/ kynurenine) and kynureninase (ratio of 3-hydroxyanthranilic acid/ 3-hydroxykynurenine), proxied by ratios of serum concentrations.

Individuals with manic symptoms showed a shift towards a higher KMO activity ($\chi 2 = 7.14$, $Df = 2$, $p = 0.028$), compared to euthymic as well as depressed individuals. There were no differences between the groups regarding activity of KAT and kynureninase. Within the group of depressed patients, HAMD and KAT showed a significant negative correlation ($r = -0.41$, $p = 0.036$), displaying lower metabolism in the direction of kynurenic acid.

Depression severity in bipolar disorder seems to be associated with a decreased synthesis of putative neuroprotective KYNA. Furthermore, higher KMO activity in currently manic individuals indicates an increased inflammatory state within bipolar disorder with more severe inflammation during manic episodes. The underlying pathophysiological mechanisms of the

different affective episodes could represent parallel mechanisms rather than opposed processes.

Development of an HPLC method for neopterin

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Pteridines are nitrogen containing heterocyclic compounds widely distributed in nature, exerting a number of different functions that are important for a variety of biological processes. The "unconjugated" pteridine neopterin, which biosynthetically derives from guanosine triphosphate (GTP), is of special relevance for the human immune system. In the course of the immune response, enzyme GTP-cylohydrolase 1 (GTP-CH-I) is activated in various cells. This results in the accumulation of 7,8-dihydroneopterin, which is mainly converted to neopterin in human macrophages and dendritic cells. Neopterin concentrations provide diagnostic information on the degree of immune activation in patients, and its concentrations can be analysed in different body fluids.
Since the high performance liquid chromatography (HPLC) measurement of neopterin in protein containing specimens still causes problems and the enzyme linked immunosorbent assay (ELISA) represents the standard measuring method for these fluids in routine diagnosis, this study focuses on developing a new HPLC method with fluorescence detection for the analysis of neopterin in serum and plasma. To prevent contamination of the column, proteins were removed by an acidic methanol precipitation and separation of the samples was performed using a Chromolith RP-18e column and a phosphate buffer acetonitrile gradient, yielding a good correlation between the neopterin concentrations of the HPLC method and the ones of a certified ELISA.

Vitamin D: Science versus reality

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The lipid soluble vitamin D mediates the mineralization of the bone and prevents osteoporosis. It regulates the calcium hemostasis, the phosphate metabolism, and the synthesis of the calcium transporter osteocalcin and matrix-GLA-protein (vitamin K2 dependent). Moreover, VitD is an important genetic and epigenetic modulator influencing the expression of up to 2000 genes. A plethora of publications reported involvements of VitD in the modulation of immune response, in muscle metabolism and brain/CNS functions, and a preventive role in cardiovascular and neoplastic diseases has been suggested. Recommendations for VitD "therapies" exploded in the last decade. Hence, a clear assessment of facts based on robust analytical methods and critical meta-analysis of effects is urgently needed for evidence based medicine.

The analytical reference value for VitD is calcidiol [25(OH)D3]. Calcidiol levels are stable in the circulatory system and not submitted to frequent fluctuations. As needed, calcidiol is changed to active VitD hormone (1,25-dihydroxy-vitamin-D3, calcitriol). Different immunoassays of different quality and mass spectrometry approaches exist for the measurement of VitD but analytical standardization programs are still missing. Moreover, seasonal fluctuations have been neglected in many studies. Hence, no reliable meta-analysis is available which tells the clinician clearly what to do.

Clinically, a VitD deficit is abolished if plasma values >20 ng/ml are achieved. Nevertheless, many researchers recommend higher levels >35ng/ml and postulate a latent deficit between 20 and 35 ng/ml. The statistic Austria database provides by the nutritional report of 2012 an overview of the VitD status in the Austrian population exclusive children below one year. Although this report states a deficit of VitD support in school children and elderly, calcidiol serum levels were only slightly decreased in these groups with value between 10 and 20 ng/ml. The alkaline phosphatase was within the age related reference limits and no symptoms of rickets were observed.

A deficit of VitD during growing favors osteoporosis in older ages. Babies need 400 IE - 500 IE VitD per day. Mother milk contains only 60 IE/Liter. Thus, for babies substitution is mandatory, irrespective of breast feeding or not. Premature infants must receive 1000 IE/d, mature babies until the first year of life 400 IE (= 1 drop Oleovit

D3/d, and until the age of 2 years 400 IE/d from September until May). These are explicit medical indications. For the elderly a balanced nutrition with 1 to 2 fish meals/week and proper sun exposition is sufficient enough. It is important to avoid sunscreens with protection factor > 30 because they block the production of active VitD completely.

Concerning cancer and cardiovascular diseases all preventive recommendations of VitD therapies cannot be supported. Although VitD levels are decreased in these diseases, a substitution is contra productive because the deficit is sequel and not cause of the disease. A substitution may even do more harm than benefit in these diseases.

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Neopterin as biomarker of comorbidity, toxicity and outcome in cancer patients

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Biomarkers currently play an indispensable role in the management of cancer patients. Biomarkers are used throughout the course of the disease starting with diagnosis, staging, prediction of response to treatment and prognosis, during the treatment and subsequent follow up. While most attention has been historically focused on the biomarkers associated with the tumors cells, it is now evident that *hitherto* mostly neglected biomarkers reflecting the host response to tumor growth are at least of equal importance. The host immune response to cancer is a crucial determinant of outcome in cancer patients. For example, in breast cancer patients treated with preoperative chemotherapy, the presence of lymphocytes in the tumor is one of the strongest predictor of pathological response. Cancer incidence increases with age, and the decision of tumor management in individual patients is often complicated by the presence of comorbid conditions that could eventually harbor a prognosis even more serious than the cancer itself. Neopterin is a biomarker of cellular immune response that is known to be increased across a spectrum of different malignant tumors. Elevated neopterin concentrations are not specific for cancer, but the production of neopterin is increased across a range of different diseases, including, for example, atherosclerosis and associated disorders. While at first glance this non-specificity may seem to be a disadvantage, the fact that neopterin is increased in other chronic disorders may paradoxically be of advantage in the assessment of overall prognosis of cancer patient. Serum and urinary neopterin concentrations are increased by therapy, including systemic administration of cytotoxic drugs or irradiation. It has been demonstrated that in patients with rectal carcinoma treated with chemoradiation, neopterin concentrations correlate with circulating levels of citrulline, a biomarker of gut dysfunction and also with the volume of irradiated bowel. Higher neopterin concentrations were associated the presence of complications. In conclusion, the determination of neopterin in the serum or urine may be used not only a complex predictor of prognosis that also covers other comorbidities, but also as a biomarker and predictor of treatment side effects in cancer patients.

Modulation of kynurenine pathway by probiotic bacteria in domestic chickens

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Kynurenine (Kyn) is the major break down molecule of tryptophan (Trp).Trp is also neurotransmitter precursor by being processed along the serotonin pathway. Altered serotonin neurotransmission is associated with abnormal pecking behaviour towards conspecifics in birds kept for egg-laying. Additionally, feather-pecking birds were shown to have a decreased population of *Lactobaccillacae*

in their gut when compared to non-peckers. Trp metabolism modulation by lactic bacteria is reported in mammals, where specific *Lactobacillus* bacteria exerted positive effects on stress-induced anti-social behaviours. We investigated the effect of a probiotic supplement (*Lactobacillus rhamnosus JB-1*) on plasma concentrations of Trp and Kyn in adolescent laying hens. Eighty-six birds (19 weeks of age) were assigned to 6 probiotic groups (P) or 6 control groups (C) (7 birds / group). P birds orally received 5*109 *Lactobacillus rhamnosus JB-1*™ dissolved in 1 mL of drinking water and received 1mL of water through the same procedure every day during 5 weeks. Plasma concentrations of Trp and Kyn were determined before and after treatment. Generalized linear mixed models were used to assess the effect of the supplementation on concentrations of Trp, Kyn and Kyn/Trp ratio.

From puberty to egg laying, we observed a decrease in Trp and Kyn concentrations (P <0.05), which was accentuated in the P groups. P birds showed an 11% decrease in Trp and 29% decrease in Kyn (P <0.05) and 18% decrease of Kyn/Trp (non-significant) over time.

Findings demonstrate that *Lactobacillus rhamnosus JB-1*™ was successful in modulating Trp and Kyn levels during the beginning of lay, which is a major life-event in laying hens. A follow-up experiment will investigate the potential of these bacteria to modulate plasma Trp and Kyn levels in order to prevent stress-induced anti-social feather-pecking behaviour.

Tryptophan degradation in cancer

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The definition of “evading immune destruction” as an emerging hallmark of cancer reflects the increasing recognition of immune suppression and escape as critical traits of malignancy. The degradation of tryptophan is a potent immunosuppressive mechanism involved in the suppression of anti-tumor immunity. The expression and activity of the tryptophan degrading enzymes indoleamine-2,3-dioxygenase (IDO) or tryptophan-2,3-dioxygenase (TDO) are upregulated in tumors or in the tumor microenvironment. The talk will give an overview of tryptophan degradation in cancer, its regulation, the molecular mechanisms involved in its effects, ways to measure tryptophan metabolites and the development of drugs that modulate this pathway.

Adverse outcome pathways: OECD’s transforming science interface and its needs

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Adverse Outcome Pathways (AOP) describe how toxicological effects may propagate from molecular initiating events (MIEs) to key events (KEs) at the cellular level and KEs at the tissue and organ level up to adverse outcomes at the organism and population level. The AOPs´ purpose is to build a mechanistic basis for the proper use of *in silico* and *in vitro* methods. AOPs shall be described in a standardized and comprehensive format. As such, AOPs may be useful for many scientific fields [1], and may provide an applied working field with an interface to regulatory authorities or industry. The AOP work is governed by an OECD Expert group and freely available via the OECD´s AOP-knowledge base [2]. Currently there is a critical bottleneck for the scientific review of AOPs and this could be an opportunity for academia and journal editors to become involved. Moreover, the AOP concept requires the comparison of uncertainties of the current *in vivo* reference data with those of the newly introduced methods [3]

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Plasma and urine neopterin concentrations in a healthy individual during weight loss caused by diet

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This case study investigated the influence of the anti-inflammatory agent Wobenzym 10 (Mucos Pharma, Unterhaching, Germany) and of strict diet with cabbage soup on urine and serum neopterin concentrations. Urine (collected daily between 6:00 – 6:30) and serum (collected once weekly between 8:00 – 8:30) were monitored daily and were collected under defined conditions in a 64 year old male during one month (23-Jan-2019 – 21-Feb-2019). Samples were collected in the morning and were processed within two hours of collection, aliquoted and then frozen. In total 24 samplings of urine and 13 serum samplings were feasible. During the collection phase, dietary habits of the volunteer changed two times: A one week period of normal was followed by daily intake of Wobenzym and thereafter followed by a strict diet with cabbage soup.
Serum and urine neopterin were measured by ELISA (BRAHMS, Hennigsdorf, Germany), blood count was measured on equipment from SYSMEX (Kobe, Japan), nutrition parameters urea, creatinine, protein, albumin, transferrin on VISTA 1500 from SIEMENS (Vienna, Austria).

Average neopterin concentrations in the <run-in> period were 24.0 nmol/L followed by 23.2 nmol/L in the Wobenzym phase and dropped to 19.2 nmol/L during cabbage days. Absolute urine neopterin concentration behaved in a similar way beginning with 116, and continuing with 100 ($p < 0.05$) and 90 nmol/L ($p < 0.01$), neopterin to creatinine ratios also dropped from 209 to160 and thereafter increased to 233 µmol/mol creatinine. Body weight was stable during the run-in (94.7 kg) and Wobenzym phase (94.7 kg), but declined under cabbage soup (92.5 kg; $p < 0.01$).

The volunteer lost (some) weight, while at the same time serum neopterin went (slightly) down. The urine neopterin to creatinine ratio seemed to be lower in the Wobenzym-period, and its effect could have persisted into the diet period. Thus, when doing the next experiment, wash-out periods (1 month?) seem necessary. The decline of neopterin as an immunological marker could relate to the beneficial effect of weight loss.

Sleep duration and timing in relationship to *Toxoplasma gondii* IgG seropositivity and serointensity

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Evidence links *Toxoplasma gondii* (*T. gondii*), a neurotropic parasite, with schizophrenia, mood disorders and suicidal behavior, all of which are associated and exacerbated by disrupted sleep. Moreover, low-grade immune activation and dopaminergic overstimulation, which are consequences of *T. gondii* infection, could alter sleep patterns and duration. Sleep data on 833 Amish participants [mean age + SD = 44.28 + 16.99 years; 59.06% women) was obtained via self-reported questionnaires that assessed sleep problems, duration and timing. *T. gondii* IgG was measured with ELISA. Data were analyzed using multivariable logistic regressions and linear mixed models, with adjustment for age, sex and family structure.*T. gondii* seropositives reported less sleep problems ($p < 0.005$) and less daytime problems due to poor sleep ($p < 0.005$). Higher *T. gondii* titers were associated with longer sleep duration ($p < 0.05$), earlier bedtime ($p < 0.005$) and earlier mid-sleep time ($p < 0.05$). Limitations include cross-sectional design, and unclear direction of causality, if any. It seems unlikely that sleep mediates the previously reported associations between *T. gondii* and mental illness. Future longitudinal studies

with objective measures are necessary to replicate our findings. Improved sleep, earlier bedtime and tolerating poor sleep in seropositives is consistent with possible immunoregulatory effect of *T. gondii* as a "microbial old friend". It could also temporarily increase the fitness of the host, thus potentially protecting longevity and exploratory capacity of the intermediate host to increase potential of exposure to the permanent host and, thus, transmission of the parasite.

This project was supported by the University of Maryland, Joint Institute for Food Safety and Applied Nutrition and the U.S. Food and Drug Administration (U.S. FDA) through their cooperative agreement FDU.001418 (PI, Postolache). Additional support was received from the Mid-Atlantic Nutrition Obesity Research Center (NORC) Pilot & Feasibility Project (Postolache, PI), a sub-award of the parent grant P30DK072488 (Simeon I. Taylor, Program Director) and the Merit Award 1 I01 CX001310-01 from CSR&D/Veterans Affairs Administration (PI, Postolache).

Porphyromonas gingivalis IgG K serotypes, and estimates of anhedonia and hopelessness/ dysphoria in the Old Order Amish

Postolache TT, Wadhawan A, Ryan KA, Daue ML, Constantine N, Dagdag A, Makkar H, Brenner LA, Lowry CA, Hoisington, A. Gulati A, Gurman S, Bui Q, Ernst RK, Reynolds MA

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Chronic periodontitis is an important cause of inflammation predictively implicated in metabolic and cardiovascular disease, and more recently in neurodegenerative disorders, such as Alzheimer's and Parkinson. The most common pathogen causing chronic periodontitis is *Porphyromonas gingivalis* (*P. gingivalis*), known to have the capacity to form biofilms, evade and manipulate immune cells, and translocate to the arterial tree, the gut, and from the gut, to the liver. It induces overproduction of cytokines like IL-1β that contribute towards this tissue destruction and generalized low-grade inflammation. *P. gingivalis* has clonal types that participate in periodontal infections express serologically distinct surface antigens. Antibodies against K1 and K6 serotype-specific capsular carbohydrate K antigen- were previously related to virulence periodontitis (Simms et al 2001, Laine et al 1998, van-Winkelhoff et al, 1993). *P. gingivalis* individual subjects are typically infected with just one or two *P. gingivalis* clonal types of the total of 32 that had been isolated (Ali et al 1997). As chronic periodontitis was previously associated with depression, we investigated if K IgG antibody titers are positively associated with cardinal symptoms of depression, and if any of the more virulent serotypes have stronger links. Participants were 880 Old Order Amish – Wellness study, age (SD) 44.5 (17), 59% women, were recruited via the Amish Wellness Research Project in Lancaster PA. IgG G Titers to purified K antigen from strains W50, HG184, A7A1–28, 49417, HG1690 and HG1691, representing serotypes K1-K6 as well as a recently discovered serotype K7 were measured by ELISA in plasma derived from fasting blood samples, based on a modification of the protocol of Califano et al., (J.Peridontol, 1999). Mood was estimated on PHQ 2- with specific answers for anhedonia and hopelessness-dysphoria, current and ever. Log titers were analyzed as continuous variables. Seropositivity for each of the seven serotypes was defined as having a logtiter value higher than the mean plus two

standard deviations of logtiters for a specific serotype of *P. gingivalis*. Statistical methods included linear and logistic regression, after adjustment for age and sex. Among the participants 1.3% reported current anhedonia, 2.9% current hopelessness- dysphoria-, "current either" 4.0% and ever either 12%. Current anhedonia and "current either" were significantly associated with logtiters for K6 and K7, current dysphoria with logtiters for K6 and "ever either" with K7 ($p < 0.05$ for all). For seropositivity, K6 was significantly associated with current anhedonia ($p < 0.005$), and positivity for any serotype with "current either" anhedonia and hopelessness-dysphoria ($p < 0.01$).

In conclusion, K6 positivity, previously linked with pathogenicity and virulence in periodontitis in humans and animal models, has been found most consistently associated with cardinal depression symptoms. Limitations are the cross-sectional design, and the direction of causality, if any, and the use of a limited self-report mood scale that only estimates mood symptom. Strengths are lower sample heterogeneity and virtually no smoking, a major confounder in periodontal disease. Our results support the need for randomized trials of periodontal treatment for mood disorders in patients with K6 and 7 high titers for *P. gingivalis* and/ or evidence of moderate/ severe periodontal disease.

Supported by the University of Maryland School of Dentistry, Baltimore, MD, with additional support Rocky Mountain MIRECC, MVA-Core and NORC, NIDDK/ NIH/ Bethesda Maryland.

Changes in tryptophan metabolism during psychiatric rehabilitation

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In recent decades a number of studies have shown an association between the Tryptophan (Trp)-Kynurenine (Kyn) axis and neuropsychiatric disorders. However, the role of the Trp-Kyn pathway on the affective status in a general psychiatric cohort requires clarification. This study aimed to measure peripheral changes in Trp, Kyn and the Kyn/Trp-ratio as well as Trp-catabolites in individuals undergoing a 6-week course of intensive treatment program. Sex specific changes (1), response to treatment (2) as well as clinical parameters (3) and associations with Trp breakdown were analyzed.

For the current report, 598 individuals with lifetime affective disorder completing a 6-week rehabilitation program were included.

1) In both sexes, psychiatric symptoms decreased significantly over time. There was a significant difference between women and men regarding the changes in Trp, Kyn and Kyn/Trp over time even if controlled for relevant covariates (multivariate: $F(3.369) = 3.036$, $p = 0.029$, $\eta^2 = 0.024$). Kyn as well as Kyn/Trp concentrations increased significantly in men over time (Kyn: $z = -2.077$, $p = 0.038$; Kyn/Trp: $z = -2.307$, $p = 0.021$).

2) In the extreme groups of treatment non-responders (n=39) Kyn increased significantly while the Kyn/Trp ratio decreased significantly in the excellent treatment responder group (n=48) over time. A significant "treatment response group" x "time" interaction was found for Kyn [$F(1.82) = 5.79$; $p = 0.018$] and the Kyn/Trp ratio [$F(1.85) = 4.01$, $p = 0.048$].

3) Changes in Kyn as well as Trp-catabolites hydroxykynurenine and kynurenic acid correlated significantly with changes in the body mass index over time.

Discussion: During the course of psychiatric treatment Trp breakdown is different between women and men, as well as depending on treatment response. Sex specific changes in Trp breakdown might also influence treatment response. Importantly, changes in body mass index were associated with changes in Trp breakdown which strengthens the thesis of shared pathophysiological pathways between somatic comorbidities (as adiposity) and affective disorders.

AGMO and etherlipids in 3T3-L1 adipocyte differentiation

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Alkylglycerol monooxygenase (AGMO) is the only known enzyme capable of metabolizing ether lipids such as alkylglycerols and lyso-alkylglycero-phospholipids in a tetrahydrobiopterin dependent manner [1]. These lipids are exclusively synthesized in specialized compartments, the peroxisomes. Knockout mice deficient in etherlipids are infertile and have defects in brain structuring and eye development [2]. We know from our experiments that AGMO is expressed in murine adipose tissue and the 3T3-L1 preadipocyte cell line. However, the precise physiological role of alkylglycerols and AGMO in several developmental processes including adipocyte conversion is not as well understood as compared to their ester analogues.

Herein we studied the role of AGMO by knocking it down and submitting 3T3-L1 to adipocyte differentiation and analyzed the lipid content during adipogenesis in cells with modulated AGMO activity compared to control cells. Furthermore, we looked at the expression of several early, intermediate and late adipocyte specific genes to address signal routes influenced by AGMO and its substrate. We will present our current results and propose a possible mechanism how AGMO is involved in *in vitro* adipocyte conversion of 3T3-L1 cells.

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The health impact of exposure to disinfection by-products in indoor swimming pool – looking at immunological biomarkers

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Swimming practice is highly recommended due to numerous health benefits, but involves exposure to disinfection by-products (DBP), such as trihalometanes (THM), known to be associated with adverse health effects. Bromodichloromethane, dibromochloromethane, bromoform and chloroform, belonging to the chemical family of THM, are among the most abundant and toxic DBP that are generally detected in the chlorinated water. With data obtained in the frame of the HEBE project, the present study aims to investigate the association between exposure to different THM and immune activation and inflammation in the swimmers, by analysing a set of biomarkers.

For this purpose, THM water concentrations were obtained from 9 indoor chlorinated swimming pool facilities located in the North of Portugal. Data from bromodichloromethane, dibromochloromethane, bromoform and chloroform will be used to estimate exposure doses through different routes using Swimmer Exposure Assessment Model2 (SWIMODEL). Plasma concentrations of neopterin, tryptophan, kynurenine, phenylalanine, tyrosine as well as kynurenine/tryptophan and phenylalanine/tyrosine ratios will be analysed as immune biomarkers from 221 swimmers.

The preliminary information here obtained will be useful to perform a human health risk assessment associated with swimming pool exposure to THM. Taken together this data, risk management strategies should be developed in order to minimize THM exposure, without compromising disinfection efficiency and swimming benefits.

This work was supported by Project NORTE-01-0145-FEDER-000010, Health, Comfort and Energy in the Built Environment (HEBE), cofinanced by Programa Operacional Regional do Norte (NORTE2020), through Fundo Europeu de Desenvolvimento Regional (FEDER).

Alkylglycerol monooxygenase in *Dictyostelium discoideum*

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Alkylglycerol monooxygenase is a tetrahydrobiopterin dependent enzyme which cleaves alkylglycerols and alkylglycerol phospholipids at the sn-1 position to yield a fatty aldehyde. It is the only enzyme known to degrade 1-O-alkyl ether lipids. Its occurrence is in general limited to bilaterial animals.

Dictyostelium discoideum is a cellular slime mold which is widely used as a model organism to study cellular differentiation. It belongs to the phylum *Amoebozoa*, intraphylum *Mycetozoa*, and due to its cooperation of single cells to a multicellular fruiting body it is also called social amoeba. We have found an alkylglycerol monooxygenase reading frame in Dictyostelium discoideum with significant similarity to the human enzyme. Overexpression of this reading frame increased alkylglycerol monooxygenase activity, while its knockout abrogated the enzymatic activity. Dictyostelium discoideum is peculiar in that it synthesizes tetrahydrodictyopterin as major pteridine which is a diastereomer of tetrahydrobiopterin. Dictyostelium discoideum alkylglycerol monooxygenase, but not rat liver microsomal alkylglycerol monooxygenase, preferred tetrahydrodictyopterin over tetrahydrobiopterin as a cofactor.

Cellular differentiation, chemotaxis, and phagocytosis of fluorescently labelled yeast were not influenced by the lack of alkylglycerol monooxygenase. When grown on *Klebsiella aerogenes* bacteria, however, alkylglycerol monooxygenase deficient mutant strains showed lower growth and lower production of spores. This was not observed when the cultures were grown on axenic media which are free of bacteria.

Our results suggest that tetrahydrodictyopterin rather than tetrahydrobiopterin is used by Dictyostelium discoideum alkylglycerol monooxygenase as cofactor and that the alkylglycerol monooxygenase reaction helps *Dictyostelium* discoideum to feed on bacteria.

Resonant breathing based HRV-biofeedback training and its effects on the interaction of autonomic nervous system activity, cellular immune activation and depressive symptoms in adults. An intervention study

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Increasing evidence indicates that low-grade inflammation seems to play a crucial role in the pathogenesis of depressive symptoms. Additionally, disturbed autonomic balance can be found in depressed individuals, manifesting itself in lower vagus nerve activity. Vagus nerve stimulation (VNS) has shown promising results in improving depression, although its mediating mechanisms are still unclear, the vagal anti-inflammatory reflex might constitute a possible pathway. Objective: This study tries to elucidate whether resonant breathing RVBiofeedback (RB), a non-invasive way of VNS can modulate cellular immunity and if the latter mediates the effect of vagal activity on the magnitude of depressive symptoms.

92 inpatients of a psychiatric rehabilitation program were randomly assigned to either the experimental (EG) or waitlist control group. Additionally, to the standard rehabilitation protocol the EG received weekly guided breathing sessions and was instructed to practice RB twice daily for 10 minutes. RB was supported by a portable biofeedback device (Qiu, Biosign GmbH; Ottenhofen, Germany), aiming at facilitating breathing training. Participants filled out the Beck-Depression- Inventory II, completed a short-term heart rate variability (HRV) recording before and after the 5-week period. Neopterin was measured from participants' urine at three time points: twice before and once after the rehabilitation period (RP).

There was an increase in neopterin from pre to post rehabilitation $F(2, 1205.12) = 3.19$, $p < 0.05$, $\eta p^2 = 0.042$, with no moderating effect of the breathing intervention.

Additionally, a positive correlation between neopterin and depressive symptoms was found pre but not post rehabilitation ($r = 0.283$, $p < 0.05$). Depression was decreased in both groups, although to a greater degree in the EG ($p < 0.05$, $\eta p^2 = 0\ .056$). Changes in neopterin did not predict the improvement of depressive symptoms. Further, no associations between linear HRV parameters and neopterin were found.

The results suggest that RB might not modulate cellular immunity, indicating that other mechanisms may drive the beneficial effect of VNS on the recovery of depressive symptoms. Additionally, the inconsistent trajectories of neopterin and depressive symptoms over the course of the 5 week study period illustrate the complex role of immunological processes in depression.

Novel links between kinase signaling and tryptophan metabolism

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The phosphoinositde-3-kinase (PI3K) – mammalian/mechanistic target of rapamycin (mTOR) network is a central regulatory hub that senses nutrients, growth factors and cellular energy, and in response controls virtually all metabolic processes in cells and organisms. Using cell biology, biochemical, and systems approaches, we explore novel links between mTOR and Tryptophan catabolic pathways and the cancer-relevant outcomes of this interplay.

Molybdopterin-modeling: the synthesis of pterin dithiolene ligands

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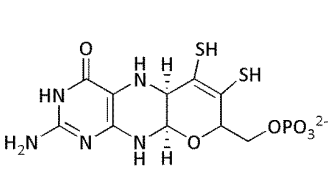
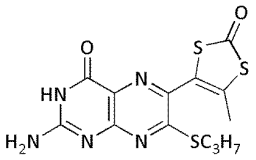
The synthesis of pterin-dithiolene ligands was achieved by employing the radical nucleophilic substitution, i.e. the so-called "Minisci- Reaction" [1]. This protocol was used for the first time by Professor W. Pfleiderer on pterin substrates [2] and proved a powerful method for the preparation of 6 acyl-pterins in course of this work. Subsequent construction of the dithiolene ring facilitates the synthesis of pterin-dithiolene ligands with completely unprotected pterin moiety (Figure below).

The molybdenum cofactor is probably one of the most relevant discoveries in the recent history of pterin chemistry and biochemistry. Many efforts have been made for the preparation of compounds able to mimic the features of the Moco ligand system called "Molybdopterin" (MPT, Figure below). In fact, the study of MPT models enables a deeper understanding of the "mechanism of function" of this cofactor and most importantly, lays the foundation for a potential treatment for the Moco related diseases MoCOD and iSOD [3].

Acknowledgements: https://cordis.europa.eu/result/rcn/179750_en.html



MPT molybdopterin or pyranopterin



pterin-dithiolene ligand

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Comparative analysis of pharmacokinetics and therapeutic efficacy of new oral and intravenous iron preparations in anemia of chronic disease (ACD)

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ACD is the most prevalent anemia in hospitalized patients. Infections, inflammatory disorders and malignancies

are the principal disease types underlying ACD. It can be aggravated by Iron Deficiency Anemia (IDA) and the correct diagnoses of patients with ACD+IDA is essential since they have contrasting therapies in comparison to those only affected by ACD. There are many different types of iron preparations available for therapy, including oral and intravenous (i.v.). Here we use an established rat model of ACD to study the pharmacokinetics, therapeutic efficacy and possible off target effects of the different iron supplementations.

Female Lewis rats were inoculated with PG-APS by intraperitoneal injection. After 2 weeks, treatment was initiated with three different types of iron: oral Ferrous Sulfate, oral Ferric Maltol and intravenous Ferric carboxymaltose. After 28 days the experiment was terminated and data analyzed.

Concerning hemoglobin levels and erythropoiesis there was no difference between the treatment groups and control. There was no important difference concerning iron absorption between the groups in the spleen, the major organ where macrophage iron accumulation takes place in ACD. Liver and duodenum showed higher absorption in the group that received i.v ferric carboxymaltose. Hepcidin, the master iron regulator, had also higher mRNA expression in the liver of the i.v. carboxymaltose injected group, being the opposite of the mRNA expression of DMT-1 and ferroportin.

The different iron preparations used had no therapeutic efficacy on our ACD model, since there was no improvement of the anemia and no use of iron for erythropoiesis in the bone marrow. The differences in the iron concentration and protein quantification in liver and spleen among the groups suggest that the oral iron preparations are not being absorbed by the enterocytes in the duodenum, probably because of the blockage by hepcidin (via ferroportin internalization and degradation). Interestingly, the i.v. ferric carboxymaltose, that doesn´t depend on iron intestinal absorption, had also no therapeutic effect in our experiment, culminating in higher hepcidin mRNA expression and iron accumulation in the duodenum.

Emerging physiological roles of alkylglycerol monooxygenase

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Etherlipids remain an elusive class of lipids because their functions are not satisfactorily understood and their metabolism still contains several orphan enzymes. For one such orphan enzyme, alkylglycerol monooxygenase (AGMO), we discovered the sequence in 2010 [1]. AGMO is the only enzyme capable of cleaving the saturated ether bond yielding free fatty acids and glycerol derivatives. Its physiological role is not well understood but we could show that it is expressed and active in mouse macrophages and is strongly up-regulated in alternatively activated macrophages [2] pointing to a potential role in immunology. Recent reports on genetic predisposition of humans for leishmaniasis and tuberculosis indeed suggest that AGMO may be relevant for the clinical course of human infectious diseases.

We have found that AGMO is expressed and active also in murine adipose tissue and in 3T3-L1 fibroblasts, a common model for adipocyte differentiation. Modulation of AGMO dysregulates 3T3-L1 adipocyte differentiation affecting also the regulation of major adipogenic markers and signaling molecules.

We will discuss the importance of AGMO for human physiology in light of our own results in macrophages and adipocyte differentiation together with findings from literature.

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Linkage of iron homeostasis to immune function and pteridine biology

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Iron is a key element in many biological processes and a stringent control of iron homeostasis is essential for life [1]. In this way iron is also essential for growth and proliferation of several microbes, but in addition iron exerts subtle effects on cell mediated immunity including

macrophage effector pathways, interferon-g (IFN-g) activity and the expression of various enzymes like inducible nitric oxide synthases (iNOS) in macrophages [2]. Thus, the control over iron homeostasis is of central importance for the course of an infection.

Inflammation thus leads to diversion of iron trafficking resulting in iron retention in macrophages and the subsequent development of anemia of chronic disease (ACD). This inflammation driven disturbances of iron homeostasis are coordinated by a collaborative work of acute phase proteins (hepcidin) and cytokines. Consequently, ACD appears as an immune driven disease [3, 4] which emerges from an evolutionary principle in order to withhold iron from circulating microbes. In addition, reduced iron availability and anemia strengthens immune response via impaired expression of the anti-inflammatory cytokine erythropoietin (EPO) and/or by amelioration of pro-inflammatory immune effector pathways as a consequence of iron deficiency. In patients the degree of anemia was inversely linked to immune activation and neopterin concentrations [5, 6], neopterin levels correlated positively with ferritin and inversely with circulating iron levels in serum. Finally the degree of anemia was inversely linked to immune activation and neopterin levels in patients with infectious diseases or cancer [5, 6]. Iron also alters the balance of Th_1–Th_2 type immunity [7] and dietary iron supplementation in regions with a high epidemic burden of infectious diseases is associated with an increased mortality from infections [8].

Pteridines can contribute to the diversion of iron traffic in ACD and directly impact on iron homeostasis. NO formation and iNOS mRNA in activated murine macrophages are regulated by iron [9]. Thereby, tetrahydrobiopterin (BH_4) impacts on cellular iron regulation via the iron responsive element/iron responsive protein (IRE/IRP) system upon stimulation of nitric oxide ($NO^{\cdot}$) formation [10]. BH_4 depletion or GTP-cyclohydrolase 1 (GTP-CH) knock out impairs T-cells proliferation, and there will be several mechanisms underlying the reduced proliferation in BH_4 deficient T-cells, as was shown by gene expression profiling. There exists a link between pteridines biology and iron homeostasis in regard to T-cells, when T-cell activation induces GTP-CH expression, GTP-CH deletion reduces BH_4 levels in stimulated T-cells [11, 12]. No effects on early activation of $CD4^+$ cells via T-cell receptor (TCR) stimulation between wild type and GPT-CH deficient cells were observed.

BH_4 increases expression of ferritin and mitochondrial iron genes but reduces cytoplasmic iron levels. BH_4 does not affect iron regulation via IRPs or via $NO^{\cdot}$ formation in activated T-cells. Inhibition of BH_4 formation by a sepiapterin reductase inhibitor reduced BH_4 formation and improves colitis in a $CD4^+$ cell transfer model. BH_4 deficiency causes impaired mitochondrial function which can be overcome by iron supplementation. BH_4 increases oxygen consumption rate of cells (OCR) and dose dependently reduces cytochrome Fe^{+3} to cytochrome Fe^{+2}.

Both, iron deficiency and genetically driven iron accumulation impair mitochondrial function. BH_4 is needed for full activation of $CD4^+$ cells which is linked to iron metabolism and mitochondrial respiration as well as to control of radical formation and mitochondrial oxidative stress. Inhibition of BH_4 formation reduces pro-inflammatory pathways driven by $CD4^+$ cells and improves disease activity in models of auto-immunity. Activation or stimulation of BH_4 enhances $CD4^+$ activation and improves anti-tumor immunity in experimental models. BH_4 can overcome the inhibitory effect of kynurenine on $CD4^+$ activation [12]. Thus, the regulatory network between pteridine biology, iron homeostasis and immune function of macrophages and CD4 cells emerges as an interesting field of research with implications on multiple inflammatory diseases.

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