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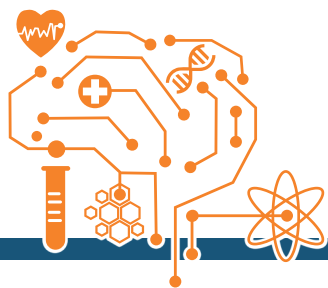
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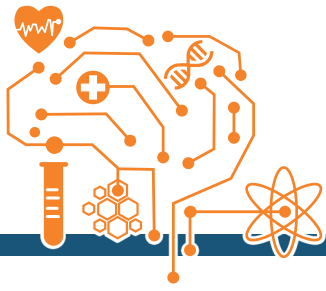
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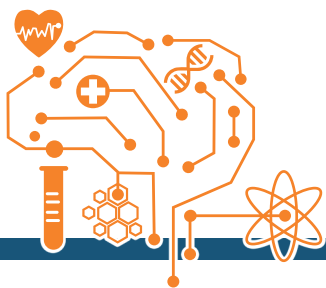
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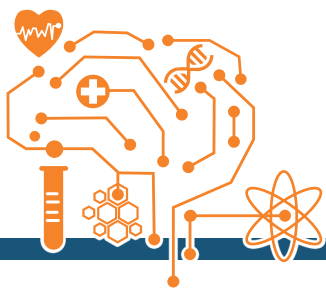
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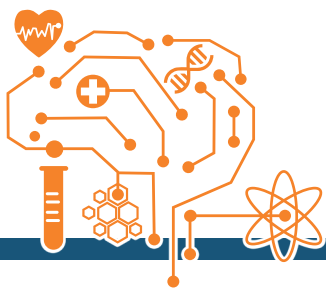
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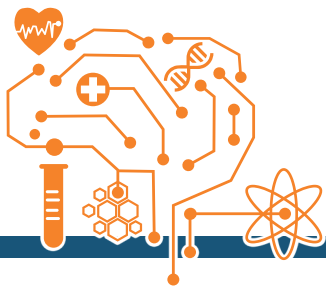
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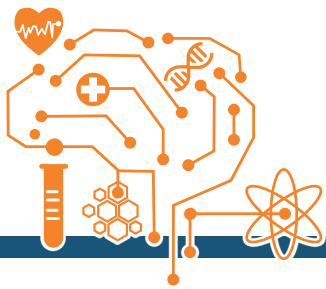
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BIOLOGIA CELULAR E MOLECULAR



Altered extracellular purines is associated with blood biomarkers in critical COVID-19: a time-course study

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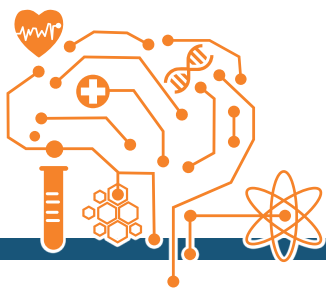
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Background: Previous study from our group have reported that some alterations in the metabolism of extracellular purines can contribute to COVID-19 severity. In fact, purinergic signalling may contribute concomitantly to both uncontrolled inflammatory response and immunosuppression. Although severe COVID-19 patients have lower adenosine levels, the course of adenine-based molecules during hospitalization is unknown. **Objective:** To evaluate the systemic levels of extracellular purinergic molecules, hematological, clinical immunology index and biochemical markers, as well as the expression of ectonucleotidases CD39 and CD73 in CD4+ and CD8+ T cells in controls, non-ICU COVID-19 and ICU COVID-19 patients. **Methods:** Blood samples were collected from non-COVID pneumonia controls (n = 10) and COVID-19 patients at hospital admission (T1). Additional blood samples were collected from survivors and non-survivors COVID-19 patients at the discharge time from the hospital (T2: 0 to 72 h before leaving hospital or death). Plasma samples were used for adenine-based purine determinations by liquid chromatography-tandem mass spectrometry (LC-MS/MS). We also analyzed hematological variables (leukocyte, neutrophil, lymphocyte, and platelets counts), clinical immunology index (SII index, NLR, and PLR) and clinical biochemical markers (urea, LDH, CK, CK-Mb, lactate). The expression of CD39 and CD73 in T cells were determined by flow cytometry. **Results:** At hospital admission, lower ATP and increased AMP was identified in non-COVID-19 infection, non-ICU COVID-19 and ICU COVID-19 compared to healthy controls. ICU COVID-19 patients also presented lower ADP and adenosine levels than healthy controls, while non-ICU COVID-19 patients had lower adenosine levels compared to controls. The evaluation of hematological variables revealed lower lymphocyte and platelet counts in ICU COVID-19 patients compared to non-ICU group, which resulted in higher SII index and NLR in the former group. Indeed, ICU COVID-19 group presented higher LDH and CK-Mb activities than non-COVID infection. The lower extracellular levels of ATP and adenosine suggest impaired ectonucleotidases

activity to metabolize adenine-based purine molecules. The incubation of PBMC obtained from a healthy donor with plasma obtained of healthy control (n=5) and ICU COVID-19 groups (n=5) revealed that the plasma from ICU COVID-19 patients leads to increased expression of CD39, PD-1 and KLRG-1 concomitant with lower CD73 and CD127 expression in CD4+ T cells of a health donor. Furthermore, ICU COVID-19 plasma incubation leads to CD8+ T cells and lower CD73, CD28 and CD127 and higher PD-1 and KLRG-1 expression compared to the trials with plasma of healthy controls. Deceased COVID-19 patients presented decreased ATP levels concomitant to higher adenosine concentrations at T2. Indeed, significant differences in delta values of both ATP and adenosine compared to ICU COVID-19 survivors. Moreover, increases in extracellular adenosine is a surprising result identified in deceased ICU COVID-19 group at T2. Previously data pointed out that sepsis is characterized by a strong immunosuppression via adenosine-inhibiting innate antimicrobial activity. Here, we identified a higher proportion of deceased ICU COVID-19 patients with sepsis who developed sepsis (66,7%) compared to those who survived with sepsis (30,8%). Deceased COVID-19 patients with sepsis presented higher adenosine levels compared to survivors COVID-19 group and survivor COVID-19 with sepsis individuals. Interestingly, the incubation of a PBMC obtained from a healthy donor with plasma of COVID-19 patient with sepsis leads to lower phagocytic capacity and phagocytic index of *C. Albicans* and lower phagocytic index of *S. aureus* compared to no sepsis COVID-19 plasma trial. **Discussion/Conclusion** These results indicate that the modulation of purinergic signaling may occur in conjunct with exhausted low-stimulatory T cell phenotype in severe COVID-19. Moreover, in COVID-19 patients with sepsis, phagocytic capacity and phagocytic index for bacteria and fungi seem to be compromised. Taken together, our results indicate that purinergic signalling may be altered during the course of hospitalization and contribute to secondary infections.



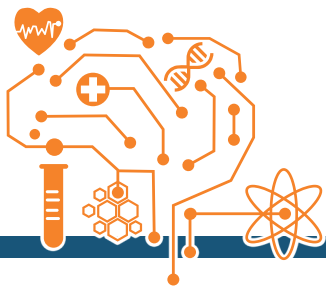
Analysis of E-NPPs in different glioblastoma models

DESTRO, Júlia¹; SILVEIRA, Priscila dos Santos¹; WINK, Márcia Rosângela¹; MARSON, Isabele Iser¹

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Introduction: Gliomas are the Central Nervous System primary tumors and they result from an uncontrolled proliferation of glial cells, reaching the highest degree of malignancy in the form of Multiform Glioblastoma (GBM). At this stage, the GBM has less delimitation, tumor recurrence, infiltrative tendency, and, in some cases, due to its high degree of cellular heterogeneity, chemoresistance and increased angiogenesis, which results in few patients survival. In the tumor microenvironment, it has been studied the role of extracellular signaling of the Purinergic System, which is composed of nucleotides and nucleosides, receptors and ectoenzymes. Among them, one that is little investigated is the Ectonucleotide Pyrophosphatase/Phosphodiesterase (E-NPPs), the type II transmembrane glycoproteins with hydrolysis capacity and important physiological functions. **Objective:** To analyze the expression of the E-NPPs enzymatic family in different models of glioblastomas. **Methods:** For this, we used models of different levels of complexity, such as monolayer GBM cell culture and three-dimensional model using spheroids in three different strains of human

GBM: U87, A172 and U251. The research is still in progress and in order to evaluate the functionality of the enzymes their activity will be measured, as well as the expression at the mRNA and protein level by immunohistochemistry. **Results:** The preliminary results are the spheroids cultured with 5.000 and 10.000 cells, for 5 and 10 days, which will be submitted to immunohistochemistry for analysis of purinergic system antibodies. **Discussion/Conclusion:** In this way, it is expected to contribute to the understanding of the action mechanism of the purinergic system enzymes that play a role in the metabolism of extracellular nucleotides in glioblastoma pathology. A better understanding of the components that act in the tumor environment may promote the development of new therapeutic resources or increase the effectiveness of treatments already used. **Acknowledgments:** To teacher Márcia Wink for never giving up on me and to Priscila Silveira for having patience teaching me all the procedures and lab conduct. Also, a very special thanks to all the biocell lab group for the support and company.



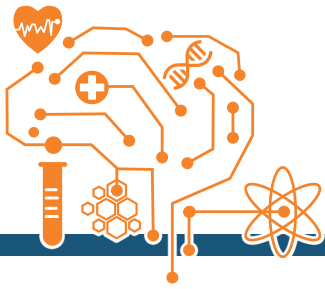
Development and characterization of a novel bioink containing decellularized spinal cord tissue for 3D bioprinting for neural tissue engineering

GARRIDO, Marcelo^{1,2}; FRANÇA, Fernanda Stapenhorst²; PRESTES, João Pedro²; SOMMER, Luiz²; SPERLING, Laura-Elena³; PRANKE, Patricia²

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Over the last few years, 3D bioprinting has emerged as a promising approach in the field of tissue engineering. This technique allows for the production of 3D scaffolds to support cell transplantation due to their ability in mimicking the extracellular environment, a key feature for achieving functional recovery. Using 3D bioprinting, it is possible to functionally recover tissue with poor intrinsic regeneration capacity, such as nervous tissue. The biomaterial structure, together with its components, drive cell fate and dictate the ability to regenerate damaged tissue. Decellularized extracellular matrix can be used as a bioink component to enhance cell adhesion, survival and proliferation. The aim of this study was to produce a bioink using lyophilized rat Decellularized Spinal Cord Tissue (DSCT) for 3D bioprinting. Rat spinal cord tissue was collected, cut in 1 cm length segments and decellularized using 1% sodium dodecyl sulfate (SDS), 1% Triton X-100 and PBS in a 9 hour protocol. To confirm the decellularization, genomic DNA (gDNA) was quantified by spectrophotometry, and histological sections of the samples were stained with DAPI or with hematoxylin and eosin. Immunohistochemistry analyses were performed to evaluate the presence of specific neural cell proteins. MTT cytotoxicity assay was used to analyze DSCT cytocompatibility, using DSCT extract or direct contact of the cells with DSCT. The bioink was produced with 1.5% lyophilized DSCT, 4% alginate, 3% gelatin and a cell density of 1.5×10^6

cells/mL using PC12 cells, a widely employed neural model. The bioink was used to print a cube with an Octopus extrusion-based bioprinter, and each construct had approximately 50 μL . Rheological characterization was performed using a rheometer with Peltier equipment. Cell viability was analyzed using MTT assay 1 and 3 days after bioprinting. DNA quantification indicated a 50-fold DNA reduction, with retention of less than 250 ng/mg of wet tissue. Histological staining indicated the presence of only a few cells. Immunohistochemistry analyses showed a reduction in GFAP, NF-M, TUJ and laminin in the DSCT compared to the control. MTT cell viability assay showed that the decellularized matrix is not cytotoxic for PC12 cells. The hydrogel presented shear thinning behavior and low G''/G' ratio, allowing for good printability without compromising cell viability after 3D bioprinting. 1 day after printing, the samples showed a 50% viability reduction due to the stress the cells are submitted to during bioprinting. However, this effect was reversed 3 days after printing, with a 50% increase in cell viability. To our knowledge, this was the first bioink described containing DSCT. The bioprinted material could not only maintain cell viability but also stimulate cell growth on a 3D structure. According to the data mentioned above, this bioink represents an easily-available biomaterial suitable for neural tissue engineering.



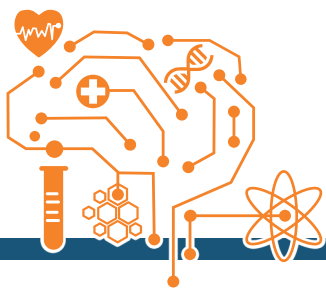
Evaluation of cannabidiol on brain dysfunction in experimental sepsis: relationship with PPAR γ receptor activation

MACHADO, Richard Simon^{1,2}; GAVA, Fernanda Frederico²; CARLI, Raquel Jaconi¹; JOAQUIM, Larissa Silva^{1,2}; MATHIAS, Khiany^{1,2}; STORK, Solange de Souza^{1,2}; DANIELSKI, Lucinéia Gainski²; CALDARDO, Vanessa²; GENEROSO, Jaqueline da Silva²; BARICHELLO, Tatiana²; BITENCOURT, Rafael Mariano¹; REZIN, Gislaine Tezza¹; PETRONILHO, Fabricia Cardoso²

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Introduction: Sepsis is characterized as a life-threatening organ dysfunction due to a dysregulated host response in the presence of infectious pathogens. Neuroinflammation and oxidative stress are pathophysiological mechanisms involved in Sepsis-associated Encephalopathy (SAS), which is related to brain damage and memory impairment. Cannabidiol (CBD) has been widely studied due to its anti-inflammatory and immunomodulatory properties. **Objective:** To examine whether CBD exerts a neuroprotective effect in experimental sepsis, and if the effect is dependent on the activation of PPAR γ using a specific receptor antagonist. **Methods:** Male *Wistar* rats (250-350g) were subjected to cecal ligation and perforation (CLP) sepsis model with SHAM animals as control. Animals were divided into SHAM+vehicle, CLP+vehicle, CLP+CBD (10mg/kg), CLP+antagonist of PPAR γ , GW9662 (1mg/kg), CLP+CBD (10mg/kg)+GW9662. Ten days after the CLP and CBD treatments, the Passive Avoidance Behavioral Test was performed, and later the prefrontal cortex and hippocampus were collected to perform

biochemical analyses: Myeloperoxidase (MPO), Nitrite Nitrate (N/N), Catalase (CAT), Thiobarbituric Acid Reactive Substances (TBARS), Carbonyl Protein. **Results:** CBD reduced the MPO activity in CLP+CBD group compared to the CLP vehicle. Treatment with PPAR γ inhibitor alone or in combination did not attenuate MPO activity. There was a reduction in N/N concentration, lipid peroxidation, and oxidative damage to proteins in the CBD-treated group. In the Passive Avoidance Behavioral Test, the SHAM+vehicle and CLP+CBD groups had an increase in latency (training vs. Test sessions) 24h later. **Conclusion:** CBD was effective in improving the parameters of oxidative stress and neuroinflammation, and improving aversive memory performance of animals from PPAR γ receptor activation. **Acknowledgments:** To Funding agencies CAPES, CNPq. To the University of Southern Santa Catarina. To the laboratory of Cerebrovascular Diseases from University of the Extreme South Catarinense. To the lab members who helped in all stages of the project. **Keywords:** Sepsis, Cannabidiol, oxidative stress, PPAR γ



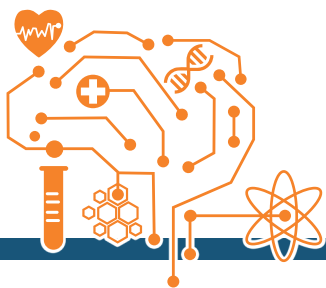
Hydrogel containing decellularized spinal cord tissue induces M2 polarization in macrophages

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Tissue engineering (TE) is a multidisciplinary research field that aims to promote regeneration, remodeling and growth of damaged tissues using biomaterials, bioactive molecules and/or cells. The use of 3D bioprinting as a promising approach in TE due to its ability to produce biomaterials with a well-defined 3D structure and a controlled bioink composition. However, the introduction of a bioprinted material into an organism can lead to an inflammatory response that can lead to the biomaterial degradation and enhance tissue destruction. Macrophages play a central role in controlling inflammation and when exposed to a stimulus it polarizes into a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype. In this context, the aim of this study was to evaluate the macrophage polarization (M1 and M2) and inflammatory response when exposed to a hydrogel containing Decellularized Spinal Cord Tissue (DSCT). RAW 264.7 macrophages were used to conduct the experiments. The hydrogel was produced using sodium alginate, gelatin and decellularized spinal cord tissue (DSCT) of rats dissolved in 0.9% NaCl solution containing HEPES to a final concentration of 4%, 3% and 1.5%. To evaluate inflammatory response the cells were cultured in a 48 well culture plate with standard medium (negative control), stressed with LPS or hydrogen peroxide (positive control) or in contact with the hydrogel. Reactive oxygen species (ROS) production was measured by 2,7-dichlorofluorescein diacetate (DCF-DA) oxidation and MTT cell viability assay was used to measure cellular metabolic activity. Pro-inflammatory cytokines IL-1 β e IL-6 and anti-inflammatory cytokine IL-10 was quantified by ELISA, following manufacturer's specifications. To access

antioxidant defenses sulfhydryl compounds were quantified by spectrophotometry. To evaluate the expression of cell markers CD45, CD69 and CD206 associated with M1 e M2 phenotype, flow cytometry was performed. DCF results showed no difference in ROS production comparing the groups with different hydrogel compositions to the control. MTT assay indicated a viability reduction of approximately 60% in the hydrogel group compared to the negative control. Cytokines quantification indicated that the hydrogel group secreted more IL-6 (165.3 pg/mL) compared to the negative control (110.84 pg/mL) and positive control (145.48 pg/mL). The cytokines production increase in the hydrogel group also occurs in IL-10 secretion. The cells exposed to the hydrogel secreted more IL-10 (28.12 pg/mL) than the negative control (20.57 pg/mL) and positive control (22.66 pg/mL). Regarding IL-1 β secretion, the hydrogel group secreted more IL-1 β (59.20 pg/mL) than the positive control (131.9 pg/mL), but showed an increase compared to the negative control (56.66 pg/mL). The biomaterial group presented a higher sulfhydryl compounds production compared to the negative and positive control. Flow cytometry analyses indicated a 40% increase in CD 206 expression, a classic M2 polarization marker, in the hydrogel group. Furthermore, the cells exposed to the hydrogel presented a 30% reduction in CD 45 and CD 69 expression compared to positive and negative control. These results indicated a M2 polarization after cultivating the cells in contact with the hydrogel. Moreover, the aforementioned data indicates that the biomaterial does not induce an inflammatory response.



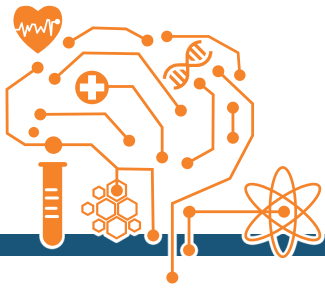
Peripheral oxidative stress in systemic infection after ischemic stroke

JOAQUIM, Larissa¹; VIANA, Rodrigo¹; CARDOSO, Beatriz Steiner¹; DANIELSKI, Lucinéia Gainski¹; MATHIAS, Khiany¹; MACHADO, Richard Simon¹; STORK, Solange¹; GAVA, Fernanda Frederico¹; FARIAS, Brenno¹; LANZZARIN, Everton Venicius Rosa¹; BERNARDES, Gabriela Costa¹; STRICKERT, Yasmin Ribeiro¹; NOVAIS, Lineiro¹; DACOREGIO, Carlos¹; BONFANTE, Sandra¹; DIETZE, Wendel¹; BITENCOURT, Rafael Mariano¹; PETRONILHO, Fabricia¹

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Introduction: Ischemic Stroke (AVCi) represents 87% of all stroke, and in Brazil already reached 222.773 thousand individuals. AVCi has a greater effect in women than in men, then women have more events and are less likely to recover. The pathophysiological mechanisms of AVCi determine events such as neuroinflammation and oxidative stress, leading the individual to a systemic immune suppression, being sepsis one of them. The role of oxidative stress associated with inflammatory conditions is well characterized, event that can be observed in organs such as brain, heart, lung, spleen, liver, kidneys. **Objective:** To evaluate the relationship between AVCi and sepsis through oxidative parameters in male rats submitted to the animal model of AVCi and sepsis. **Methodology:** Male Wistar rats aged 60 days were randomized into groups: SHAM+SHAM, SHAM+MCAO, SHAM+CLP, MCAO+CLP, and submitted on day 0 to Middle Cerebral Artery

Occlusion (MCAO) and on day 7 submitted to Cecal Ligation and Perforation (CLP) technique, suffering painless death after 24 hours and the heart and liver were removed for biochemical analysis. **Result:** We observed that sepsis exacerbated reactive oxygen and nitrogen species in animals that suffered AVCi as well as increased sensitivity of lipid peroxidation in sepsis. The brain damage caused by AVCi induces protein oxidation and the association of two conditions was effective to potentiate a decrease of antioxidant enzyme catalase decrease. **Conclusion:** Sepsis can potentiate brain oxidative stress in female rats undergoing AVCi. **Thanks:** CAPES. University of Southern Santa Catarina. Laboratory of Cerebrovascular Diseases of the University of Extremo Sul Catarinense. Behavioral Neuroscience Laboratory. The team members who helped in all stages of project. **Keywords:** ischemic stroke, sepsis, oxidative stress, neuroinflammation.



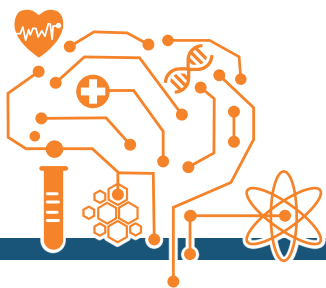
Protection of maternal immune activation by lipopolysaccharide on brain oxidative changes in the offspring after sepsis in adulthood

STORK, Solange^{1,2}; GAVA, Fernanda Frederico²; MATHIAS, Khiany^{1,2}; MACHADO, Richard Simon^{1,2}; JOAQUIM, Larissa^{1,2}; FARIAS, Brenno¹; STEINER, Beatriz¹; ABRAHÃO, Sabini¹; CIDREIRA, Thainá¹; DANIELSKI, Lucinéia Gainski²; SANTOS, David²; PETRONILHO, Fabricia²

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Introduction: Sepsis is considered an infection associated with organ dysfunction due to a host's dysregulated response. Approximately 17 million people develop sepsis each year worldwide. In sepsis, the exacerbated production of pro-inflammatory mediators and the release of free radicals amplify the damage to the host. Lipopolysaccharide (LPS) is a component of the outer membrane of gram-negative bacteria. **Objective:** To evaluate whether maternal immune activation during gestation, using LPS, can modulate sepsis neuroinflammation in the adult offspring and result in protection against oxidative brain changes. **Methodology:** Male *Wistar* rats were randomized into saline+sham, saline+CLP, LPS+sham and LPS+CLP. For the characterization of the LPS animal model, pregnant females were randomly divided into two groups according to the experimental protocol, one exposed and one control. Sepsis was induced using the cecal ligation and perforation (CLP) technique when the offspring turned 60 days old. After 10 days, the animals underwent painless assisted death and the prefrontal cortex and hippocampus

were collected for biochemical testing. **Results:** We observed increased myeloperoxidase activity and neutrophil infiltration in the brain structures of sal+CLP and LPS+CLP rats. However, we found that LPS preconditioning in the prenatal period can decrease oxidative damage to proteins and lipids in brain structures after acquiring sepsis in adulthood. **Conclusion:** Maternal immune activation, induced by LPS in the prenatal period exerted a neuroprotective role by decreasing oxidative stress to proteins and lipids in adult rats. In addition to elevating catalase enzyme activity in these animals. We also saw that the production of ROS, ERNs and neutrophil infiltration in the prefrontal cortex and hippocampus was increased. **Acknowledgements:** To the Funding Agencies CAPES and CNPq. To the University of Southern Santa Catarina. To the laboratory of Cerebrovascular Diseases of the University of the Extreme South Catarinense. To the lab members who helped in all stages of the project. **Keywords:** sepsis, lipopolysaccharide, brain, oxidative stress.



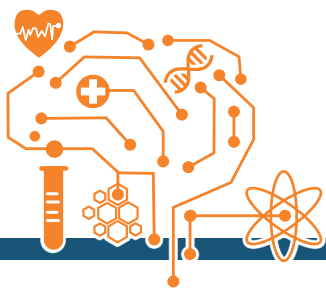
Pure iron implant produced by metal injection molding : a promissor biomaterial for cardiovascular stents

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Background/introduction: Metal injection molding (MIM) has become an important manufacturing technology for biodegradable medical devices. Biodegradable metallic materials are expected to corrode gradually *in vivo* after providing structural support to the tissue during its regeneration and healing processes. Pure iron is a promising biodegradable metal for use in stents due to its mechanical properties and biocompatibility. In previous work, we produced pure iron samples by MIM using a new eco-friendly feedstock from natural rubber (*Hevea brasiliensis*) and observed their biocompatibility *in vitro* with Mesenchymal Stromal Cells and *in vivo* after subcutaneous implant in *Wistar* rats. **Objective:** In this step of the study, our objective is to develop a prototype of a biodegradable cardiovascular stent produced by this process. **Methods:** Iron samples were processed by MIM, and after injection, the hardness and metallographic structure were evaluated, as well their biocompatibility with Human Umbilical Vein Endothelial Cells (HUVECs). The indirect cytotoxicity assessment was performed according to ISO 10993. HUVECs were cultured with different iron extracts for 24 and 72h and their viability was evaluated by MTT assay. The cytomorphology after indirect culture was analyzed after DAPI and Phalloidin staining by fluorescence microscopy. In addition, the adherence and morphology of the HUVECs on the surface of iron implants were observed by SEM (Scanning

electron microscopy). **Results:** The manufactured samples have adequate physical, and mechanical characteristics for biomedical devices. HUVECs did not alter their viability, morphology, and adherence in contact with iron. **Discussion/conclusion:** Cells showed good compatibility with iron extracts. According to ISO 10993-5, if viability is reduced by >30% in comparison to the control, the biomaterial has a cytotoxic potential. There was no significant change in cell viability, so iron is cytocompatible. Cells cultured with iron extracts exhibited typical morphology of the HUVECs, which preserved the integrity of the actin cytoskeleton and nuclei after iron extract exposition. The HUVECs adhered and did not change their morphology when cultured on the iron surface. Therefore, pure iron produced by MIM can be considered a promising material for stent application. It has the physical and mechanical properties necessary to encourage intravenous tests in preclinical models. **Acknowledgments:** This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001; FAPERGS/MS/CNPq/SESRS n. 03/2017 – PPSUS 17/2551-0001 (Process: 416-2, Marcia R. Wink and 413-8, Lírío Schaeffer); FAPERGS/CNPq/SEBRAE 08/2019 – PDEmp 20/2551-0000207-1 (Diego P. Wermuth) and CNPq MS-SCTIE-Decit/CNPq n° 12/2018 (441575/2018-8).



Relationship of ischemic stroke and experimental sepsis on brain oxidative parameters

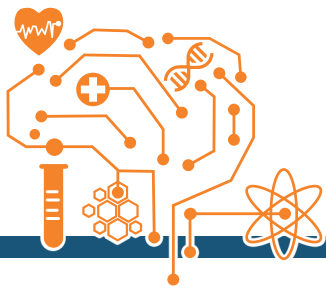
MAURILIO, Khiany Damaris Mathias^{1,2*}; JOAQUIM, Larissa Silva^{1,2}; CARDOSO, Beatriz Steiner¹; DANIELSKI, Lucinéia Gainski²; STORK, Solange de Souza^{1,2}; MACHADO, Richard Simon^{1,2}; NOVAIS, Linério¹; GAVA, Fernanda Frederico²; FARIAS, Brenno¹; BERNARDES, Gabriela Costa¹; LANZZARIN, Everton Venicius Rosa¹; BONFANTE, Sandra Regina Santana Aguiar¹; DIETZI, Wendel¹; PERIN, Carlos Henrique Dacoregio¹; STRICKERT, Yasmin Ribeiro¹; GENEROSO, Jaqueline da Silva²; BITENCOURT, Rafael Mariano¹; PROPHIRO, Josiane Somariva¹; PETRONILHO, Fabricia Cardoso²

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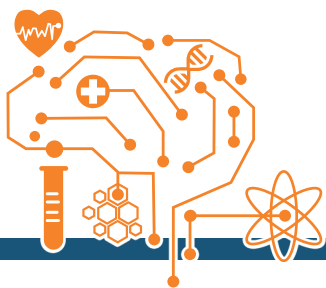
Introduction: Cerebrovascular accident (stroke) affects about 13 million people annually and is responsible for the death of approximately 5.5 million of those affected. In addition, it is estimated that 50% of survivors become chronically disabled. Ischemic stroke represents the most common type of stroke, occurring in approximately 87% of all cases. Ischemic stroke has a greater effect on women than on men because women have a greater number of events and are less likely to recover. The pathophysiological mechanisms of ischemic stroke trigger events such as neuroinflammation and oxidative stress, promoting immunosuppression in patients and making them more susceptible to the development of infectious conditions and sepsis. Consequently, oxidative and inflammatory damages can be frequently observed in brain regions such as the prefrontal cortex and hippocampus. **Objective:** To evaluate the relationship between stroke and sepsis through oxidative stress parameters in female rats submitted to the experimental model of stroke by middle cerebral artery occlusion (MCAO) and sepsis by cecal ligation and perforation (CLP). **Methodology:** 60 days old adult female *Wistar* rats were randomized into sham+sham, MCAO+sham, sham+CLP, and MCAO+CLP groups and subjected to the respective

experimental or control models. After 24 hours, the animals underwent painless assisted death, and the hippocampus and prefrontal cortex were collected for biochemical testing: Myeloperoxidase (MPO), Nitrite Nitrate (N/N), Catalase (CAT), Thiobarbituric Acid Reactive Substances (TBARS), Carbonyl Protein.

Results: Sepsis exacerbated the production of reactive oxygen and nitrogen species in animals undergoing ischemic stroke, as well as higher sensitivity of lipid peroxidation in sepsis. The brain damage caused by stroke induced protein oxidation and the association of both conditions potentiated the decrease in the activity of catalase. **Conclusion:** Pathological conditions such as ischemic stroke and sepsis can promote the breakdown in brain homeostasis through oxidative stress and when the conditions are associated, there is potentiation of brain oxidative stress in female rats. **Acknowledgments:** To the Funding Agencies CAPES, CNPq. To the Laboratory of Behavioral Neuroscience. University of Southern Santa Catarina. To the Cerebrovascular Diseases Laboratory of the University of the Extreme South Catarinense. To the lab members who helped in all stages of the project. **Keywords:** infection, cerebrovascular, oxidative stress, ischemia



EPIDEMIOLOGIA E MÉTODOS DIAGNÓSTICOS



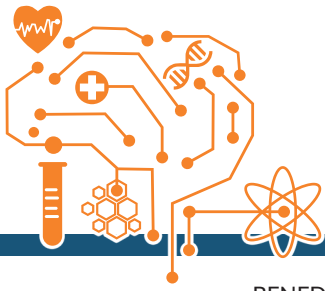
“Spiders like it hot”: The seasonality of 15 year of arachnidism in the southern state of Santa Catarina

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Introduction: Envenomation by animals, mostly terrestrial ones, is considered a public health problem in Brazil, spiders making up a large part of the animal envenomation cases. The Brazilian south, currently, is the region with the highest number of notifications, while Santa Catarina (SC) is the second state in number of registered spider bites. **Objective:** The objective of this research was to study the temporal and seasonal aspects of the notified cases of spider bites in the state of Santa Catarina, between the years 2007 and 2021. **Methods:** Data on reported accidents caused by spiders in the state of Santa Catarina between 2007 and 2021 were obtained through the Notifiable Diseases Information System (Sistema de Informação de Agravos e de Notificações - SINAN) from the Brazilian Ministry of Health (Ministério da Saúde do Brasil). Data such as month of occurrence, year of occurrence, and spider classification (*Phoneutria*, *Loxosceles*, *Latrodectus*, other genera, ignored/blank) were collected. The Kruskal-Wallis non-parametric test was used to examine statistical differences between the variables and the number of registered spider bites, before that, the data was tested for normality using the Shapiro-Wilk test. Only confirmed information were used in the tests. All data categorized as “ignored” or “ignored/ blank” was excluded. **Results:** There were 80,960 spider bites registered from 2007 to 2021, more than half (52%, n = 42,149) of those were not from Brazil’s medically important spider genera, with 15.6% (n = 12,629) of the total notifications being from blank/ignored cases

and 36.4% (n = 29,459) from other genera. The year with the most notifications was 2019 (8%, n = 6,265,) and the one with lowest being 2021 (5%, n = 4,052). No distinctive temporal pattern could be found, as the number of cases notified were homogeneously distributed throughout the years, and no statistical ($\chi^2= 9.26$, $df=14$, $p = 0.814$) difference was found when tested. The highest number of recorded spider bite accidents occurred during January (13%, n = 10,300), while July (4%, n = 3,213) had smallest number of notifications., with the difference between months showing statistical significance ($\chi^2= 31.4$, $df= 11$, $p= <0.001$). **Discussion/Conclusion:** Is notable the concentration of notifications in the summer months (south hemisphere summer). This seasonal variation shows the influence of climate on the frequency of arachnidism in SC. While the predominance of accidents in the warmer months can be attributed to the fact that spider tend to be more active in foraging during times of elevated temperatures, which can also coincide with the reproduction period of the species. This study expands on the temporal dynamics of spider bites occurrence in a hotspot for arachnidism in Brazil and it can also be applied to public health policies. **Acknowledgments:** We are thankful to the Coordination for the Improvement of Higher Education Personnel (CAPES) for the PROSUP Fee benefit of the first author and to Foundation for Support to Research and Innovation of the State of Santa Catarina (FAPESC) and CAPES for the funding for the second author.



Colonization by *Streptococcus pneumoniae*: respiratory manifestations in a vaccinated children cohort in one year follow-up

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¹ Universidade Federal de Ciências da Saúde de Porto Alegre – UFCSPA; ² Instituto Moriguchi: Centro de Estudos do Envelhecimento; ³ Universidade Federal do Rio Grande do Sul – UFRGS.
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Background/introduction: *Streptococcus pneumoniae* is a potentially pathogenic agent in children which can often transmit it to adults and elderly. It is associated with the high rate of morbidity and mortality. In order to trigger a disease, there needs to be a prior colonization of the nasopharynx. The implementation of the 10-valent pneumococcal conjugate vaccine (PCV10) has been a major advance for Brazilian health by reducing invasive pneumococcal disease (IPD). Current observations report substitution of vaccine serotypes by non-vaccine ones and its repercussion on colonization and respiratory disease is unknown. **Objective:** Investigate the influence of colonization by *Streptococcus pneumoniae* in vaccinated children for respiratory outcomes throughout one year of observation. **Methods:** This was a follow-up observational double-blinded prospective cohort study with children aged 18–59 months from Veranópolis-RS, Brazil. A total of 228 children were eligible, with collection of nasopharynx (NP) material at “zero time”. After a loss of 1.3%, 225 children completed the follow-up study, with interviews and data collection of medical records at the end of the one-year follow-up (March/19 to October/20). It should be noted that the sole NP collection occurred at “zero time” and data for respiratory disease were collected through the one-year observation period (started counting from “zero time”). **Results:** We observed a high colonization rate (64.4%) and presence of the vaccine serotype 6B (2.8% of colonized). Colonization was more predominant in male individuals. Non-vaccine serotypes (NVS), non-PCV10 and non-PCV13, were found in 57% of the colonized cohort, demonstrating the occurrence of replacement. Observed rates of pneumonia (CAP), AOM, sinusitis, upper and lower tract ARI, tonsillopharyngitis and asthma for both of *S. pneumoniae* were very similar with no verified association between pathology and

colonization. We found that children colonized by pneumococcus do not present an increased risk for respiratory diseases or antimicrobial administration. The only exception was found when we stratified the sample by age as the incidence of pneumonia was higher in the age group of 18 to 23 months in which the colonizing serotype 6B (PCV10) showed a statistically significant difference, which represents a novel observation. We found association between serotype 6B colonization and pneumonia through the one-year period, in children under 2 years. Although it is not possible to establish that the colonizing pneumococcus caused CAP due to the lack of a documented etiology and the limitations of the study design, the association between the capsular type of the vaccine and this CAP cannot be neglected. **Discussion/conclusion:** We demonstrated that carrying *Streptococcus pneumoniae* did not influence the occurrence of respiratory disease in a fully-vaccinated children population, except for CAP for children under 2 years and when colonization occurred through serotype 6B. This information is important by means of designing public policies for the National Immunization Program once vaccination is the only available tool for pneumococcal disease prevention so far. **Acknowledgements:** Centers for Disease Control and Prevention (CDC) for performing pneumococcal serotyping; Moriguchi Institute for helping us with secretariat support and logistics; Cristiane Bündchen for providing statistical assistance; Veranópolis Health Department and HCSPL (Hospital São Peregrino Lazziozi) for collecting information from medical records; PPSUS- FAPERGS for support and funding. We thank all children and their parents/guardians who took part in the study and the nurses, pediatricians and community health workers at the Public and Private Health Systems of Veranópolis.



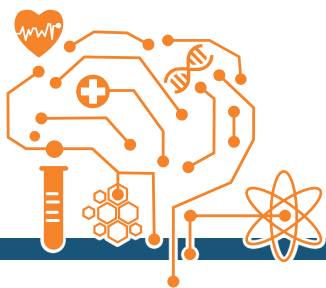
Detect-S: an mHealth application to assist health professionals to identify suicide risk in hospitalized patients

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Introduction: Suicide is a complex phenomenon that affects millions of people annually in the world. Although there are instruments, scales and some applications for the assessment of suicide risk by health professionals, there is not yet a gold standard instrument that effectively detects or drastically helps to reduce the number of deaths related to the event. From this perspective, emerged the urgency of developing a new tool, which broadly and completely assesses all psychopathological aspects of suicidality and also could be joined to an available Mobile-Health technology. **Objective:** The purpose of this study was to propose an Information Technology based model to help detect the risk of suicide by health professionals in the hospital environment. **Methods:** This is an exploratory-descriptive applied research with qualitative methods and quantitative observational cross-sectional approach regarding the use of information technology in the health domain. The method used is based on mobile app prototyping. The application development was performed using the Classic Software Development Lifecycle Model, which means that development phases were applied, such as: requirements definition, analysis, design, development, testing and implementation of the prototype. The research was approved by the ethics committees from Federal University of Health Sciences of Porto Alegre – UFCSPA (protocol number: 2,465,977), Hospital Santa Rita (protocol number: 2,739,735) and Presidente Vargas Maternal Children’s Hospital (protocol number: 2,465,977), in accordance with Resolution No. 466 of 12 December 2012 of the National Health Council. Seven health

professionals participated in the study, 1 data scientist and 3 Biomedical Informatics students, 20 patients (10 cases and 10 controls) at the Santa Rita Hospital of the Santa Casa de Misericórdia Hospital Complex in Porto Alegre and 18 patients (10 cases and 8 controls) of Presidente Vargas Maternal Children’s Hospital. **Results:** The research resulted in the development of a prototype and a new scale for suicide risk assessment that was coupled with Mobile-Health technology, to evaluate patients admitted to units in the general hospital environment, which made it a multiplatform application (iOS and Android). It covers 16 features and was called Detect-S (suicide risk detection). **Discussion/Conclusion:** Mobile Health or Mobile Health inevitably in the current scenario is a reality in the routine of health professionals, making access to patient data easier and more dynamic, contributing to the agility of actions that will be effective in being cared for. Thus, it can be concluded that Detect-S application emerges as a possible innovative and technological solution to assist healthcare professionals in the early detection of suicide risk in patients admitted to inpatient units. All this encourages us in other research in a future perspective to validate the Detect-S scale that is deployed in the prototype and expand the application of the application in other scenarios and not just the hospital environment. It is noteworthy that the prototype was filed with the patent application at the National Institute of Industrial Property (INPI) in March 2019, under case number 870190021717 e n° do INPI: BR 102019004410-1. **Keywords:** Scale; Suicide; Assessment; Questionnaire.



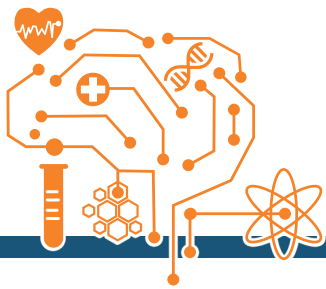
Development of a nutritional intervention program focused on the cost of food for primary health care users

FLORIANO, Jassana Moreira^{1*}; VIEIRA, Laura Morshak¹; ESCOUTO, Giselle Souza¹; ONGARATTO, Mariana Aubin¹; LONGHI, Paula Rainone¹; FAVERZANI, Natália Machado¹; ALVES, Felipe de Carvalho¹; BUSS, Caroline¹

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Primary health care users have already mentioned difficulties in having a healthy diet, due to the cost of food and insufficient knowledge. This study aimed to develop and apply a nutritional intervention program for primary health care users and to analyze the participants' perceptions about the intervention, in order to assess the program's viable points and define adaptations for future applications. The convenience sample was recruited at primary health care unit in Pontão (RS). The sample was composed of 25 participants (22 women and 3 men) aged between 19 and 69 years. Most participants had completed high school (60%). According to per capita family income (R\$ 1,587.33), the sample presented a class D economic classification. The intervention program was applied between July and September, 2021. Each meeting lasted approximately 1 hour. During the interventions, information related to the cost of food products was in accordance with a survey of these data in supermarkets in the region, in order to use real values of the food available in the town

and respect the food culture of the participants. The program was carried out in two editions. The first was conducted in a hybrid format, being in-person and synchronous online through a digital platform. Fifteen participants were invited to participate. In accordance with the safety measures of the capacity of the place, 8 in-person spots were made available per day, in addition to the possibility of online participation. Most participants (52%) reported having had a significant increase in learning about the topics. By using open questions to verify the knowledge and feelings of those involved, it was possible to identify the cultural habits and the participants' assimilation regarding the cost of food consumed by their family. The insights gained through this study allowed the development of new materials in order to improve the interest and engagement of volunteers in the course of subsequent applications of the intervention. The realization in a small town and the lack of long-term evaluation of the intervention are limitations that should be addressed in future works.



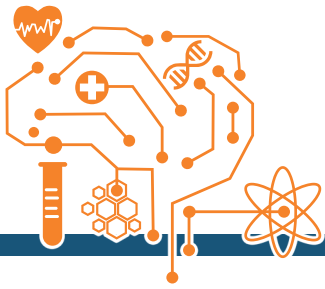
Does regular exercise impact the lung function of healthy children and adolescents? A systematic review and meta-analysis

BALBINOT, Fernanda¹; CLAUDINO, Felipe César de Almeida²; LUCAS, Pedro Kazlauckas³; MARTINS, Ana Paula Donadello³; WENDLAND, Eliana Marcia¹; GERBASE, Margaret Weidenbach¹

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Background: Several factors can influence lung function in early life with long-term consequences on respiratory health. Exercise has been shown to improve lung function in children and adolescents with respiratory diseases such as asthma and cystic fibrosis. However, evidence of its beneficial effect on healthy individuals is still unclear. **Objective:** To assess the quality of the available evidence on the effect of exercise on the improvement of lung function in healthy children and adolescents. **Methods:** We included intervention studies that examined changes in the forced vital capacity (FVC), the forced expiratory volume in the first second (FEV1), and the peak expiratory flow (PEF) of healthy children or adolescents aged ≤ 18 years, after exercise interventions. The search for relevant studies was performed on MEDLINE, EMBASE, Cochrane CENTRAL, PEDro, ERIC, PsycINFO, SciELO, LILACS, Clinical trials.gov, and EU Clinical Trials register. No restrictions for language, date of publication, or type of studies were applied for any of the databases. After qualitative analysis, within- and between-group meta-analyses were conducted using R Statistical Software. Subgroup analyses were performed according to the age of participants, and the total duration of the interventions. The quality appraisal was performed a) at the study level, using the Cochrane Risk of Bias Tool for Randomized Controlled Trials and the Quality Assessment Tool for Quantitative Studies and b) at the outcome level, using the GRADE approach. This study was conducted following the PRISMA guidelines and its protocol is available on the PROSPERO platform (CRD42018114240). **Results:** From a total of 7,942 studies, only eight met the selection

criteria and were included in the analyses. Pooled results showed significant improvements in FVC (mean difference [MD]: 0.17 L) and FEV1 (MD: 0.14 L) within the exercise groups, regardless of the age groups and duration of interventions. When including only controlled trials in the meta-analysis, the FVC, FEV1, and PEF were higher in the exercise- compared to the non-exercise group, but the differences did not reach statistical significance. There was significant statistical heterogeneity between studies. Because of methodological constraints in the available studies, the certainty of the evidence on the effect of exercise in FVC, FEV1, and PEF was rated as very low according to the GRADE criteria. **Discussion/Conclusion:** Regular exercise has the potential to improve lung function parameters in healthy children and adolescents. However, there are few studies on the subject, and they have low methodological quality. This lack of good quality evidence is a concern, particularly when we consider the current context of insufficient levels of exercise and the high prevalence of overweight/obesity in the young population, which were accentuated by the outbreak of the coronavirus disease. Therefore, it is essential that future well-designed studies are carried out to determine whether exercise can be used as a tool for the improvement of lung function development in healthy children and, as such, counterbalance the adverse effects that physical inactivity, overweight, and the COVID-19 may have on the young population's lung health. **Acknowledgment:** This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.



Looking for *Aedes aegypti*: how is the mosquito infesting the Brazilian South?

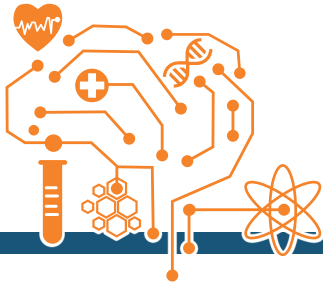
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Introduction: In Brazil, the *Aedes (Stegomyia) aegypti* mosquito is considered the main vector in the transmission cycle of the arboviruses that cause dengue, chikungunya and Zika. There are several factors related about this mosquito, such as the increase in the transmission of pathogens, linked to climatic, environmental and social factors. Southern region of Brazil has been showing an increase in dengue notifications, causing a concern about the control, monitoring and further research on the vector.

Objective: The objective of this study was analyzing the distribution of *Ae. aegypti* in the southern region of Brazil between 2003 and 2021, as well as determining the possible relationships among the incidence of this vector and the climatic and environmental variables during 2017 and 2021. **Methods:** The study analyzed the cities in the southern region of Brazil. Data from secondary sources of the Building Infestation Index (Índice de Infestação Predial – IIP) of *Ae. aegypti* from the Ministry of Health, climate data from NASA's Giovanni platform and environmental data from the MapBiomas Project were collected. In order to map the presence and absence of the vector, the data were organized using the QGIS software. The IIP data was grouped by the south brazilian mesoregions annual averages, in order to assess spatiotemporal patterns

by year, state and mesoregions. The difference among years, states and mesoregions was tested using a PERMANOVA analysis. For the purpose of assessing possible correlations between the IIP of *Ae. aegypti* with the climatic and environmental variables, the software used was Jamovi. **Results:** There was an increase in the number of IIP surveys from 2017, as well as in the number of cities that confirmed the presence of the vector. Our model was significant for four groups analyzed (south region and three isolated states). It happened a positive correlation of some environmental variables with the IIP of *Ae. aegypti*, such as urban infrastructure, precipitation maximum temperature, minimum temperature, and humidity. **Discussion/Conclusion:** In recent years, there has been a growing increase in dengue notifications in southern Brazil, with a significant increase in the number of cities infested by the *Ae. aegypti*. The correlation of climatic variables with the dynamics of the vector distribution is complex, because it can be influenced by several factors. It is noteworthy that the present work is one of the first studies that historically analyzed the survey of the IIP of *Ae. aegypti* in the cities of the southern region of Brazil, as well as the distribution of the infestation of this vector and its correlation with climatic and environmental variables.



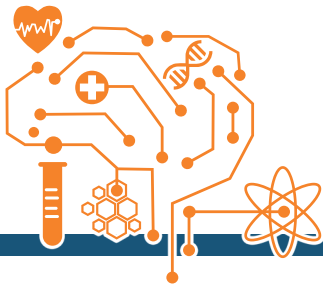
Nasopharyngeal carriage of *Streptococcus pneumoniae* among Brazilian children: interplay with viral co-infection

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Streptococcus pneumoniae is a bacterium that causes invasive diseases such as meningitis, bacteremia and especially among children, is considered one of the most serious microorganisms in childhood pneumonia, being considered one of the biggest contributors to morbidity and mortality worldwide. Respiratory viruses are the most common microorganisms that cause respiratory diseases in children. During childhood, the rate of pneumococcal colonization is high, and respiratory viruses are frequent in the nasopharynx. There seems to be a dynamic process of acquisition and elimination of various microorganisms, during which they interact with each other and with the host. As it is the site of origin of respiratory tract infections, it is of great importance to investigate the interactions in the nasopharynx, especially between pneumococcus and respiratory viruses in healthy children. A total of 230 children aged 18-59 months from the city of Veranópolis/RS were eligible. Of

these 63.9% (147/230) carried pneumococcus in the nasopharynx, the prevalence of respiratory viruses was 49.13% (113/230) and 34.8% (80/230) had co-occurrence (*S. pneumoniae* + any respiratory virus) of these microorganisms. The presence of respiratory virus was positively associated with pneumococcal colonization (54.4% vs 39.7%; $p=0.033$). Rhinovirus was the most prevalent among respiratory viruses, in 32.6% (75/230) of the study population. And there was a positive association with pneumococcal colonization (38.8% vs 21.7%; $p=0.012$). Our study contributes to the understanding of the relationship between pneumococcus and respiratory viruses. Research in healthy children is still in its very beginning, with not many studies addressing this issue. Some studies report that the presence of these microorganisms in the nasopharynx are risk factors for subsequent respiratory diseases. In the population of our study, it was not possible to reach this conclusion.



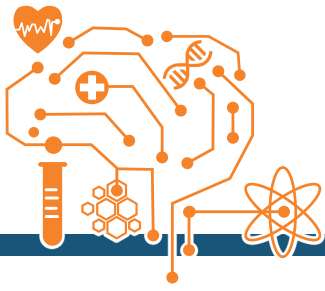
Pattern of infant mortality rates in border Sister Cities in Brazil: an ecological-type analytical observational study

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Background: The infant mortality rate (IMR) is a proxy of the living and health conditions of a given population, which allows us to assess the risk of death for children under one year. Although there is, in general, a reduction in infant mortality in Brazil [1-2], little is known about this indicator in the regions and cities located on the international borders of the Brazilian territory and the changes that occurred in the face of the migratory impact of the Americas in the period from 1996 to 2020. The objectives of this study is to assess IMR in Brazilian Sister Cities or also known as Twin Cities (municipalities that are located on the border with a large influx of people) and its social determinants over time. **Methods:** This is an ecological study, whose units of analysis were the Brazilian Twin Cities, between 1996 and 2020, based on data on births and deaths in children under one year, available in the public vital information system in Brazil. Data were identified by the city in which the infant death occurred in addition to the mother's

primary city of residence. Correlation measurements were performed to test the associations of the IMR means between the independent variables. **Results:** The Sister Cities (Bonfim, Tabatinga, Pacaraima, Porto Murinho, Cáceres, Foz do Iguaçu, Santo Antônio do Sudoeste e Dionísio Cerqueira) had higher numbers of infant deaths per place of occurrence than the number of deaths per place of maternal residence. The Northern Twin Cities exhibited the highest IMRs. Cities in the Midwest region showed variability. In the South region, most cities showed low rates. A positive correlation was identified with the Gini index with $r=0.67$ and a negative correlation with the Municipal Human Development Index indicator of $r= -0.70$. **Conclusions:** The averages of IMRs in the Twin Cities were higher than in their States. In recent years, there has been an upward trend in infant mortality in these cities. **Keywords:** Infant Mortality; Border Health; Ecological Studies.



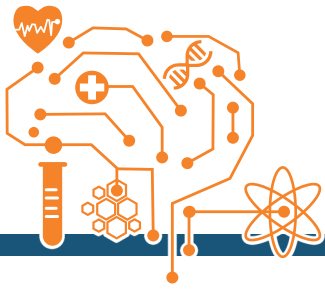
Spatial analysis of the influence of COVID-19 mitigation measures on the accessibility to diagnosis, therapy and monitoring of people living with HIV in the southern region of Brazil

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Introduction: As a result of social isolation measures, due to the risk of infection with the Sars-CoV-2 virus, responsible for COVID-19, people living with HIV may have had difficulty accessing health services during the period with more restrictive mitigation strategies. The factors that contributed to this situation were mainly the overburdening of the health care system to care for patients with COVID-19, the interruption of drug supply, fear of contamination and some health services allocating different resources (human, material, etc.) to cope with the pandemic. Thus, COVID-19 mitigation measures, although essential for the management of COVID-19, may have led to setbacks in the longitudinality of care for people living with HIV. **Objective:** To analyze whether COVID-19 mitigation measures have influenced the accessibility to diagnosis, therapy and monitoring of people living with HIV in the states of the southern region of Brazil, and, if so, the associated social determinants of health. **Methods:** Ecological, observational, human aggregate data study in geographic units (health regions) and time series (2017-2019 and 2020-2021). The unit of analysis will be the set of people living with HIV who reside in the 68 health regions of the three southern states of Brazil, which are Paraná, Santa Catarina and Rio Grande do Sul, in the period from 2017 to 2021. Georeferencing techniques will be used for distribution and spatial analysis of data. **Results:** The study expects to understand the spatial distribution of HIV-related clinical outcomes in the health regions of the southern states of the country,

in two time series, one of them being before the pandemic (2017 to 2019) and the other the mitigation period of COVID-19. In addition, to understand the difference in indicators related to clinical outcomes in the health regions and states analyzed with those recorded for the other major regions of Brazil and for the country, in both time series. Another result concerns knowing if social determinants of health are related to the rates considered of greater vulnerability to HIV (\uparrow GAP, \uparrow CD4 \leq 350, \uparrow incidence rate and \uparrow mortality rate) in the pre-pandemic period (2017-2019) and during the COVID-19 mitigation measures (2020-2021). The data will be presented in tables and thematic maps, Moran's Index will be used to analyze spatial correlation, and regression tests will be used to verify the association between variables. **Discussion:** In the United States, a study indicated that the amount of medical consultations offered to people living with HIV decreased by 33% (827), when comparing the second quarter of 2019 with the same period of 2020. In short, when compared to previous quarters, the second quarter of 2020 had the lowest number of first appointments. In India, research that sought to understand the impact of the pandemic on HIV-related service utilization pointed out that during the COVID-19 mitigation period, a significant decrease in HIV-related service utilization occurred, with a reduction to 25% of pre-pandemic levels in April/May 2020 (first wave) and a 57% reduction in April/May 2021 (second wave).



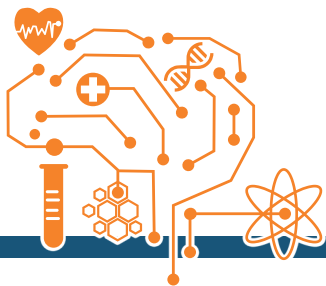
Stigmatization against patients with obsessive-compulsive disorder: an exploratory study

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Historically, people with psychiatric disorders or with evident psychopathological manifestations are a constant target of stigmatization. One of the manifestations of stigmatization is discrimination, and realizing that you are a victim of it is a stressful situation. This project aims to evaluate the concepts involved in the term stigmatization of patients with Obsessive-Compulsive Disorder (OCD), a heterogeneous and debilitating disease that affects about 2% of the population. Such concepts will be investigated through self-report measures such as experienced discrimination, internalized stigma and the perception of stigma resulting from having a psychopathology and how this influences self-esteem and adherence to treatment. The research will take place through the REDCAP Platform, which will provide a safe way for people with OCD to respond to questionnaires about sociodemographic, clinical and stigmatization data. Data collection is being done online, in the “self report” format, nationwide and disseminated through a folder on digital platforms. We plan to collect data from participants with a clinical diagnosis of OCD at the national level as a pilot study. The stigma of OCD patients seems to be one of the barriers influencing therapeutic delay. In addition, the scarcity of data on this topic makes its investigation an opportunity to

obtain more information and promote public mental health policies for this specific population. Data are being collected and measured using the following scales: Sociodemographic, Explicit Discrimination Scale, The Stigma Scale; Perceived Devaluation and Discrimination Scale; Internalized Stigma of Mental Illness Scale; Rosenberg Self-Esteem Scale; YBOCS, DYBOCS, BAI, BDI, USP-SPS; Medication Adherence Questionnaire; Quality of Life Scale - WHOQOL and SCID-V. These data are relevant so that new interventions and therapeutic alternatives can be designed to improve the quality of life of people who suffer from OCD. We are still in the data collection phase, to be completed at the end of 2022. As preliminary results, we have 60 participants who completed filling out all the questionnaires and 97 participants who partially answered them. The results analysis phase will take place after the completion of data collection. Although the conclusions and discussions can only be consolidated later, we can identify, so far, some difficulty regarding the completion of the completion of the questionnaires, probably because the format is “self report” and online. Our sincere thanks to the UFCSPA institution, participants, promoters and project collaborators.



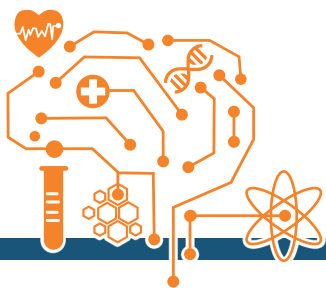
Urinalysis parameters in patients with COVID-19 presenting acute kidney injury

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Introduction: Since the beginning of the SARS-CoV-2 epidemic in 2020, studies have focused their attention on pulmonary complications, which is the main cause of hospitalizations, and is associated with a high mortality rate. In addition to respiratory system damage distress, acute kidney injury (AKI) is an important complication of COVID-19, occurring in 0.5 - 7% of cases and in 2.9 - 23% of patients in the intensive Care Unit with COVID-19, 7.58% (95% CI 3.30- 13.54%) develop AKI, with a mortality rate of 93.27% (95% CI 81.46 - 100%). **Objective:** Evaluate the incidence of abnormalities observed in the Qualitative Urine Examination of hospitalized patients with COVID-19 and relate to developmental impact of AKI and mortality. **Methods:** Patients hospitalized with SARS-CoV-2 were recruited after admission to Hospital São Camilo (Esteio/RS, Brazil) between June/2020 and December/2020. Urine samples were obtained by spontaneous urination or by catheterization and analyzed within 2 hours. Urinalysis was evaluated using reagent strips in the Urisys automated system and microscopy of the urinary sediment was performed. **Results and discussion:** 60 samples were analyzed (20 without AKI, 19 with AKI and 21 control patients - without hospitalization) and there was a prevalence of 45% (27) and 23.3% (14) of hemoglobin and proteinuria (urinary strip) with 68.4% (13) and 47.4% (9), respectively,

in patients with AKI. The presence of red blood cells in the urinary sediment was higher in patients who died compared to survivors ($P=0.041$) and was significantly correlated with mortality ($P=0.023$), with hematuria observed in all non-survivors 19 (31.7%), of which 16 (84.2%) presented AKI. In patients who died (19), the occurrence of proteinuria and hematuria was observed in 9 (47.4%) of which 7 (77.8%) had AKI, having a correlation with mortality when presented with the condition proteinuria and hematuria plus AKI ($P=0.001$). Patients with AKI are associated with mortality ($P < 0.001$). In our study, AKI was observed in 15% (3) of the survivors and in 84% (16) of the non-surviving patients, however, all non-surviving patients had hematuria, which does not allow us to observe the usefulness of this parameter to predicting the development of AKI. **Conclusion:** It is concluded that non-survivors of COVID-19 have a high incidence (84.2%) of AKI, with changes in urinary parameters (presence of blood, proteins and hematuria). It is suggested that urinalysis, together with assessment of additional biomarkers such as cytokines and stress markers in urine, may be useful in the care of patients with COVID-19 who have renal involvement, in order to identify patients at higher risk of mortality, thus guiding the allocation of care and resources. **Keywords:** Renal injury. Urinary sediment. SARS-CoV-2.



Use of food frequency questionnaires to assess dietary patterns - a Scoping Review

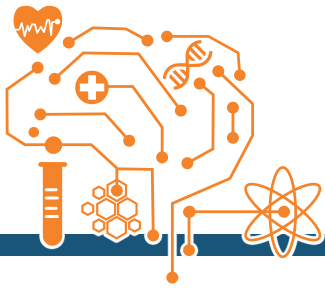
FLORIANO, Jassana Moreira¹; VIEIRA, Laura Morshak¹; ESCOUTO, Giselle Souza¹; BUSS, Caroline¹; ONGARATTO, Mariana Aubin¹; LONGHI, Paula Rainone¹; FAVERZANI, Natália Machado¹; ALVES, Felipe de Carvalho¹

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Background/introduction: The Food Frequency Questionnaire (FFQ) is a method of surveying food consumption used in epidemiological investigations to collect information on food and dietary consumption and aims to investigate the relationship between diet and disease. Defining dietary patterns through the use of food consumption tools favor the identification and association with diseases and ensure the strengthening of public policies aimed at nutrition.

Objective: To identify the existing literature that uses the FFQ as a method to assess dietary patterns in the adult population. **Methods:** scoping review study, guided by the JBI methodology and following the recommendations of the PRISMA roadmap. The search took place in September 2022, guided by the mnemonic PCC: P (Population) – Research using FFQ; C (Concept) – dietary pattern; C (Context) – adult population, based on the following guiding question “How is the FFQ used to define dietary patterns in the adult population?”. Searches were carried out on Lilacs, Scielo and Pubmed platforms, using the key terms “food pattern” and “food

frequency questionnaire” and as filtering content, publications in the last 5 years (2017-2022), written in English, were selected. **Results:** After screening, duplicate articles or articles that did not meet the specifications for an adult population were excluded. Thirty-two articles that contained information on the definition of a dietary pattern were included. The largest number of publications took place in 2019 (n=10) and the country with the most publications was China (n=8). Most studies (n=24) use the FFQ to define associations with non-communicable chronic diseases, being the most frequently identified obesity (n=3), diabetes mellitus (n=5), cardiovascular diseases (n=5), hypertension (n=5) and cancer (with different outcomes or types) (n =4). **Discussion/conclusion:** This review demonstrates the scarcity and complexity of the association of dietary patterns and consumption assessment methods. The use of semi-quantitative FFQ proves to be an option, but there is a need for further development of studies in the area.



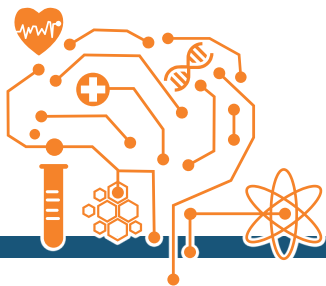
Zoonotic diseases from horses: a narrative review

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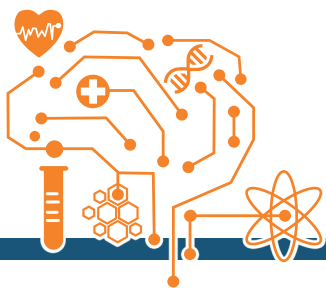
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Introduction: Zoonotic diseases have been one of the biggest concerns worldwide in the last years, since they represent a major threat, having a profound impact on global health. This concern is because in the last 25 years, 38 new pathogens have emerged and of these, 75% were originated from animal diseases. Horses are animals that live in close contact with humans and this interaction creates a potential risk to spread zoonotic pathogens. **Objective:** In this context, we sought to perform a narrative review to identify the main zoonotic diseases of horses worldwide. **Methods:** In September 2022, we searched through the PubMed platform using the keywords “equines and zoonosis”. The date range filter was from 2017-2022 (last 5 years). The research literature was restricted to studies available in English. Duplicate works and articles that were only about the molecular characteristics of the pathogens were removed. After surveying all the articles, the authors did an individual evaluation of them with a full reading to define if they served the objective of this work. **Results:** The search resulted in a total of 344 works identified in the initial search. Of these, 214 were removed for not attending the inclusion criteria, leaving 130 works for analysis. The decimal places of all results were rounded to facilitate the comparison between them and the reader comprehension. It was observed that the year with the most publications was 2020 (27%),

while 2022 was the one that presented the least (9%), however, it must be considered that for this work the searches were carried out until September 2022, with publications referring only to the previous 9 months. The most common type of study was the original article (48%), followed by reviews (14%). About the pathogens, there were articles that dealt with more than one pathogen, for this reason, the total number of the pathogens is above the value of articles analyzed (159 pathogens). The most discussed was West Nile Virus (16%), followed by Hendra Virus (11%). **Discussion/conclusion:** Both most cited pathogens are responsible for diseases that represent a serious public health problem. Hendra virus, which until then is restricted to Australia, causes an emerging zoonosis that has a high mortality rate and is classified as a level 4 biosafety organism (highest biocontainment). West Nile Virus is considered the most widespread arbovirus on the planet, in which 1 in 150 cases become neuroinvasive, inducing to encephalitis or even death. In this way, we can see that there is concern and the requirement for preventive measures to be taken, especially in the face of emerging diseases, to prevent new zoonotic pandemics from happening. **Acknowledgments:** We are thankful to the Coordination for the Improvement of Higher Education Personnel (CAPES) for the PROSUP fee benefit of the first and second authors.



FARMACOLOGIA E TOXICOLOGIA



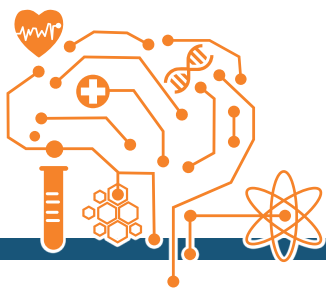
Allopurinol causes neuroprotection after experimental sepsis

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Background: Kynurenine pathway is a metabolic route of tryptophan. This pathway can be activated by degradation of tryptophan through tryptophan-2,3-dioxygenase enzyme (TDO), stimulated by exacerbated inflammation or cell stress agents. In this regard, exacerbated inflammation processes, like sepsis, can lead to overactivation of kynurenine pathway. **Objectives:** Evaluate the effects of a TDO Inhibitor administration upon the brain oxidative modifications after experimental sepsis. **Methods:** Two-month-old male Wistar rats were submitted to sepsis by cecal ligation and perforation (CLP).

Animals received Allopurinol (20 mg/kg) or vehicle via gavage once a day per 7 consecutive days. The prefrontal cortex and hippocampus were submitted for analysis and oxidative stress parameters were evaluated. **Results:** Sepsis elevated lipid and protein oxidative damage, but also compromised antioxidative enzyme functioning 24 hours after CLP. Allopurinol administration avoided prefrontal cortex and hippocampus oxidative damage on rats that survived under these parameters. **Conclusion:** Inhibition of TDO was effective in preventing oxidative stress after induced sepsis. **Acknowledgments:** FAPESC, CNPQ.



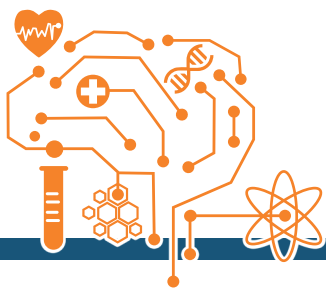
Analytical findings in plasma samples from cases of suicide attempt by self-poisoning attended in Rio Grande do Sul, Brazil

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Background/introduction: Retrogressive suicide attempt is described as the main risk factor for suicidal behavior. From this, the acute and prolonged management and treatment of patients is extremely important, prospecting the reduction of a new suicide attempts. Unlike more violent methods, which are almost universally fatal, survival following self-poisoning is common, providing an opportunity for secondary prevention, so it is important to know the main toxic agents involved as well as their use profile. **Objective:** This study aims to characterize the main substances involved in suicide attempts by self-poisoning in Rio Grande do Sul, as well as their socio-demographic profile. **Methods:** To verify recent exposure, toxicological analysis was conducted on plasma samples from patients with suspected poisoning by suicide attempt attended by the Toxicological Information Center of Rio Grande do Sul (CIT/RS) between 2018 to 2021. The analysis was performed on LC-MS/MS, performing the simultaneous analysis of 57 compounds, such as antidepressants, benzodiazepines, antipsychotics, antihistamines, anesthetics, analgesics, and illicit drugs. Information such as sex, age, place of exposure, elapsed time of exposure, severity, evolution, and day of the week was collected along with the cases. **Results:** Altogether, samples from 139 different cases were analyzed, and about 95% (n=132) were positive for at least one substance. Acetaminophen was the toxic agent with the highest incidence, being present in 67.6% of the analyzed samples. When verified by the different groups of substances, benzodiazepines and antidepressants were the most present in the samples, with about 34.5% and 25.9% respectively. Additionally, 10 cases involving illicit drugs were observed, 9 for cocaine and derivatives and one for

MDMA. Regarding the profile of the patients, 64% were female and 36% were male. The mean age of patients was 25.5±14.3 years and the age groups most frequently were 15-19 years (29.5%) and 20-29 years (35.3%). Given the place of exposure, the home was the most common, with around 90%. About 30.2% of poisonings were classified as mild, 24.5% as moderate, and 15.1% as severe. The remaining 30.2% were classified as differential diagnosis, non-toxic, or ignored. Of the 139 cases, three were fatal, evolving to death. Meanwhile, 133 were cured and three were cured with sequelae. No differences were observed in the percentages between the days of the week on which the exposure occurred. As for the time between exposure and the time to seek medical care, 24.5% took up to one hour, 54% up to 5 hours, 31.7% was equal to or greater than 6 hours, and 14.4% were ignored. **Discussion/conclusion:** The laboratory diagnosis is important in the toxicological scenario, to help direct the management and appropriate treatment of the intoxicated patient. The analytical results found in this study trace a profile of use in the population of Rio Grande do Sul that attempts suicide using medicines and/or drugs of abuse. Accessibility to substances can be considered a crucial factor in choosing a suicidal agent since the most substances detected were acetaminophen, an over-the-counter medication, and psychiatric drugs, which are commonly used in patients with a predisposition to suicidal disorders. Furthermore, demographic characteristics such as female gender and young age group were observed as predominant in the analyzed population. Finally, the data obtained serve as a basis for prospecting public policies aimed at preventing suicide and poisoning. **Acknowledgments:** CAPES; FAPERGS.



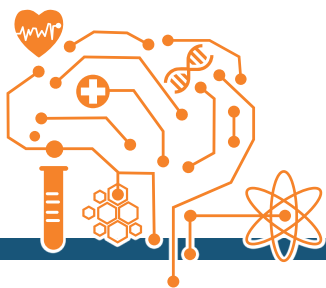
Antifungal potential of alkyl-bridged bis-imidazolium salts

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Diseases caused by fungi are very common and can be difficult to treat, thus, the development of new antifungal agents has crucial importance given the emergence of drug-resistant fungal strains and the relative scarcity of antifungals. *Candida spp.* is an opportunistic fungus in humans that acts when immunity is low causing serious diseases being associated to greater part of all nosocomial fungal mycoses in hospitals, such as bloodstream candidiasis, and respiratory, intra-abdominal, urinary tract, cutaneous and other infections. In addition, *Candida spp.* can undergo mutations leading to biofilm formation, being responsible for the protection against the immune response and antifungal agents. Recently, dicationic IS have attracted the scientific community's interest, which present greater chemical stability combined with lower levels of cytotoxicity compared to their monocationic analogues. Herein, a group of structurally complementary alkyl-bridged bis-imidazolium salts, including variations in the alkyl-bridge length (Cn: n = 3, 4, 5, 8, 10, 12) and the anion (bromide and methanesulfonate), were synthesized with the purpose of exploring their *in vitro* antifungal potential against *Candida albicans*, *C. parapsilosis* and *C. tropicalis*. The minimum inhibitory concentrations (MIC) of the alkyl-bridged bis-imidazolium were determined according to the Clinical and Laboratory

Standards Institute guidelines, document M27-S4. The bioassays were performed in triplicate with *C. albicans* ATCC 14053, *C. parapsilosis* ATCC 96144 and *C. tropicalis* ATCC 13803. The dicationic salts were tested in the concentration range from 1 to 512 $\mu\text{g.mL}^{-1}$ and the MIC values were determined after 48 h of incubation. Both *C. albicans* and *C. parapsilosis* were resistant against fluconazole, which showed a MIC of 8 $\mu\text{g.mL}^{-1}$ in *C. tropicalis*. Almost all IS presented antifungal activity with MIC \leq 64 $\mu\text{g.mL}^{-1}$ for the three species of *C. spp.*, except for **MImC3MImBr2** (MIC of 512 $\mu\text{g.mL}^{-1}$ for all yeasts) and **MImC4MImBr2** (MIC of 64-512 $\mu\text{g.mL}^{-1}$). The best antifungal profile was identified for **MImC10MImBr2**, which showed MIC of 1 $\mu\text{g.mL}^{-1}$ independent of the yeast tested. This MIC is lower compared to those obtained with the antifungal drug of choice, fluconazole, which was tested against the same isolates. This shows that these alkyl-bridged bis-imidazolium salts contain promising leads for the development of drugs against pathogenic yeasts, including resistant species. **Acknowledgments:** This study was partly financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES), Finance Code 001. The authors thank CNPq for the partial financial support.



Exposure to genotoxic substances: relationship of work in the health of the street sweeper

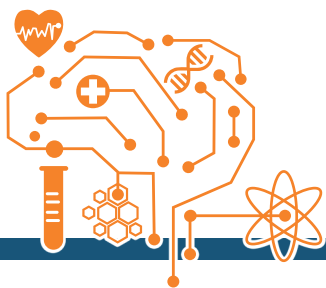
OLIVEIRA, Heluza Monteiro¹; COSTA, Edivânia Bento¹; SÁNCHEZ, Carlos Manuel Dutok¹; SOUSA, Germana Teixeira¹; COSTA, Elisandra Bento¹; SANTOS, Nathanni Queiroz¹; MORAES, Ariane Aparecida Santos¹; WETCH, Jacqueline Gonçalves Ramos¹; MENDES, Myriam Regina Zapattera¹; DIAS, Wanderson William dos Santos²

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Introduction: Exposure to toxic substances may lead to cellular alterations to the point of altering deoxyribonucleic acid (DNA), causing several systemic alterations that may lead to severe pathologies, thus, research was extremely important for the detection of Cellular changes in swamps, which are often exposed to toxic substances and unhealthy lifestyles.

Objectives: The objective of this research was to evaluate the frequency of chromosomal aberrations and nuclear alterations in oral mucosal epithelial cells as occupational risk markers due to genotoxic exposure and the relationship with work and life habits of Oiapoque swamps. **Methodology:** The study was characterized as basic, observational, quantitative analytical, exploratory, field, with laboratory and cross-sectional analysis. The research was conducted in the state of Amapá, Oiapoque, with professionals who work as street sweepers and are daily exposed to genotoxic substances in their occupational environment. **Results:** agreed to participate in the survey 28 sweepers, of which 82.14% were male; The age of 21 to 40 years represents 57,14%; Regarding the level of education, 46.43% stopped their studies in elementary school; The predominant skin color is brown, representing 78.57% of the swamps; When it comes to marital status, 46.43% are married; The predominant religion presents balance, the evangelical and catholic were represented by 42.86% each; As for the monthly salary income, 75% receive up to R \$ 1,764.00. Consume / consume tobacco 71.43%, and consumers of alcohol represent 75% of respondents. Those who have been in the profession for up to 5 years represent 39% of street sweepers; 78% of them are aware of occupational exposure;

79% report using personal protective equipment, however, there is no consensus as to their origin. 50% report receiving from the company, while 50% report buying them; The main symptoms perceived by them are vision changes, headache, diarrhea and burning / irritation in the eyes, 11% had leukoplakia, and 11% had erythroplasia; Regarding blood glucose, 47% of respondents had pre-diabetes; 25% of respondents had a hypertensive peak and 36% were overweight. Cellular findings with nuclear alterations were common mainly in the process of cell death, such as cariorexiles ($p = 0.0474$) and karyolysis ($p = 0.0473$), with statistically significant differences between men and women. In smokers and non-smokers it was also evidenced that there are statistically very significant differences ($p = 0.0020$) regarding micronuclei. In the garnishes that consume or not fruits and vegetables there are statistically significant differences ($p = 0.0269$) with pycnosis. **Conclusions:** The research showed that the street sweepers of Oiapoque are at constant risk due to occupational and occupational exposure; The appearance of lesions in the oral mucosa of the swamps was not statistically significant; The karyolysis and cariorexexis showed significant differences for the research showing significant differences between men and women and the pycnosis showing significant differences in the garnishes that consume fruits and vegetables; Micronucleus findings were statistically significant in smokers and non-smokers, showing that the onset of these changes is related to lifestyle habits. **Key words:** Genotoxicity. Gari. Occupational Exposure. Habits.



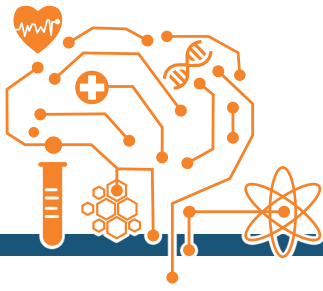
Optimization of an innovative extraction method using magnetic nanoparticles to detect cannabinoids in urine.

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Introduction: Around 144 cannabinoids have been identified in cannabis plants, among them tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) are the most prominent. Considering the cannabis control scenario and that cannabis use becomes progressively accepted, it becomes increasingly important to determine the presence of cannabinoids in biological samples. However, THC analysis represents an analytical challenge due to the low bioavailability and a well-known adherence in propylene containers that can directly affect the analysis result, impairing its determination in biological samples, promoting the necessity for innovative and applicable extraction methods. Miniaturized extraction techniques allow the use of custom sorbent phase with different types of materials including magnetic nanoparticles (MNPs). However, the use of MNPs as a sorbent for extracting psychoactive substances in biomatrices remains poorly explored. **Objective:** The aim of this work was to evaluate commercial MNPs as an extraction sorbent applied to dispersive solid phase extraction (dSPE) with a LC-MS/MS compatible methodology to the determination of cannabinoids in urine. **Methods:** The extraction method consists in mixing the adsorbent to 100 μ L of postmortem blood in a vortex mixer for 1 minute. Then, the supernatant is separated from the MNPs using a neodymium magnet, placed in a 3D printed microtube

rack, eliminating the necessity of centrifugation. After discarding the supernatant, the desorption solvent solution was added to the nanoparticles, mixed, separated and analyzed. Response surface methodologies were used to optimize the parameters that influence in this technique such as pH, MNP mass and desorption solvent. A Nexera UFLC system coupled to a LCMS-8045 triple quadrupole mass spectrometer (Shimadzu, Japan) was used for the analysis. **Results:** Acidic pH promoted a significant decrease in the analytical response based on the chromatographic peak areas. Compared to control, results were on average three times higher at pH 10 for all tested compounds. We obtained better results with higher mass of nanoparticles and the surface graphic showed that the desorption solvent with the best recovery was a mix of isopropanol, acetonitrile and methanol (1:1:1). **Conclusions:** The results inferred that MNPs are efficient to extract THC, CBN and CBD from urine in the optimized conditions. Improvements in the analytical response can be made by drying and reconstituting the extract. In addition, nanoparticles coating techniques can be used to promote a better interaction between the analytes and the MNPs. **Acknowledgments:** The authors would like to acknowledge the financial support from *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES).



The interactions between O₃ exposure and physical activity status on redox state, inflammation, and pulmonary toxicity of young men: a cross-sectional study

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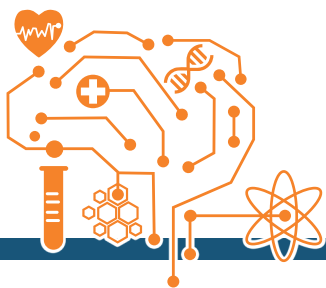
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Introduction: Exposure to traffic-related air pollutants, as NO₂ and O₃, are a crescent trend due to emissions augment and their adverse health impacts. Exercising in polluted environments could result in harmful outcomes for health and may blunt the physiological adaptations of exercise training.

Objective: This study aimed to investigate the influence of physical activity and O₃ exposure on redox status, inflammatory marker, response to stress and pulmonary toxicity of healthy young individuals.

Methods: We performed a cross-sectional study with 100 individuals that, based on their personal exposure to O₃ and physical fitness (PF) level, were distributed in four groups: Low PF + Low O₃; Low PF + High O₃; High PF + Low O₃; High PF + High O₃. We evaluated personal exposure to NO₂ and O₃, physical activity level, variables of oxidative stress (SOD, ROS, CAT, GSH, TBARS), pulmonary toxicity (CC16), and inflammatory mediators (IL-1 β , IL-4, IL-6,

IL-10, TNF- α , HSP70). Spearman correlation test to check the association among the variables was used and to compare groups we used one-way ANOVA followed by Bonferroni's post hoc and Kruskal Wallis test followed by Dunn's post hoc. **Results:** O₃ levels correlated with physical activity ($r=0.25$; $p=0.01$) but not with age or markers of body composition ($p>0.05$). The individuals with high physical fitness that were less exposed to O₃ presented higher CAT activity ($p<0.001$). **Acknowledgment:** Bruna Marmett, is supported by doctoral fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Gilson Pires Dorneles is supported by postdoctoral fellowship from (CAPES) and Alessandra Peres and Pedro Roosevelt Torres Romão are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the PQ productivity scholarship.



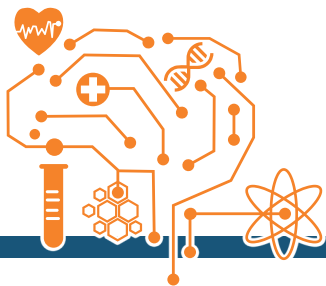
Toxicological analysis of 17 psychoactive pharmaceuticals in human hair by liquid chromatography-tandem mass spectrometry

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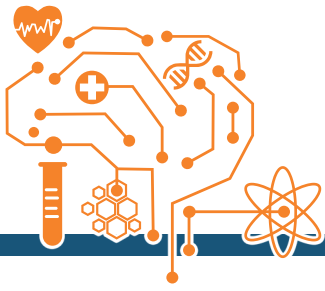
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Background: Hair testing is the main procedure for the evaluation of long-term exposure to xenobiotics since hair is a matrix that facilitates drug incorporation and has a regular growth rate, enabling a retrospective analysis. Some advantages of the use of this matrix include the larger detection window, the non-invasive collection, the possibility of storage at room temperature, and the difficulty of sample adulteration. Determining pharmaceuticals in hair generates useful data in forensic casework since information about the presence of these compounds can contribute to investigating drug-facilitated crimes and deaths. **Objective:** This study aimed to develop, optimize and validate a sensitive and specific method to determine psychoactive pharmaceuticals (alprazolam, amitriptyline, bromazepam, buspirone, carbamazepine, clonazepam, diazepam, escitalopram, fluoxetine, haloperidol, imipramine, midazolam, nortriptyline, quetiapine, risperidone, temazepam, venlafaxine, and zolpidem) in human hair samples by liquid chromatography-tandem mass spectrometry (LC-MS/MS). **Methods:** The collection of samples was performed by cutting a portion of hair in the posterior vertex region of the head. The samples were cut into segments and 20 mg were mixed with 500 μ L of methanol: ethyl acetate solution (75:25) and 6 μ L of the internal standard (0.1 μ g/mL), followed by incubation for 16 hours at 80° C. Afterwards, 10 μ L of the extract were injected in an LC-MS/MS system. Sample preparation parameters were optimized by multivariate strategies using the Statistica 8.0 software (Statsoft, USA). The type of solvent was optimized through a simplex-centroid design while the solvent volume, time of incubation, and salt concentration were by a central composite design. A Nexera-i LC-2040C Plus coupled to an LCMS-8045 triple quadrupole mass spectrometer (Shimadzu, Japan) was used for the analysis. After development and optimization, the method was validated according to the ANSI/ASB Standard O36 by the evaluation of the following parameters: lower limit of quantification (LLOQ), linearity, carryover,

endogenous and exogenous selectivity, bias, precision, and ionization suppression/enhancement. To prove the applicability of the method, real samples from the Division of Postmortem Inspection of Porto Alegre were analyzed. **Results:** The results for the optimization with the conditions representing the best responses are described in the "Methods" session. LLOQs for all analytes were 40 pg/mg and calibration curves were linear over the range (40 - 600 pg/mg) with a determination coefficient ≥ 0.99 . The method achieved satisfactory precision results for four quality control (QC) concentrations with coefficients of variation between 5.18 and 19.09 while bias ranged between -18.28 and 16.37. No carryover effects were observed, as well as no interference from endogenous and exogenous substances. Ionization suppression was significant for some pharmaceuticals such as diazepam, alprazolam, and clonazepam while others had a signal enhancement such as fluoxetine and nortriptyline. The method was applied to six authentic cases with four being positive for one or more pharmaceuticals, as follows: sample 1 - clonazepam (544.81 pg/mg); sample 2 - clonazepam (82.15 pg/mg) and zolpidem (145.20 pg/mg); sample 3 - fluoxetine (80.40 pg/mg); and sample 4 - zolpidem (338.93 pg/mg). **Discussion/conclusion:** This study presented an optimized and validated method for the quantification of 17 pharmaceuticals of different drug classes. The method's sensitivity is adequate according to the determined 50 pg/mg cut-off of benzodiazepines and z-drugs in human hair established by the EWDTs. Also, it was proved that it can be applied to the analysis of these pharmaceuticals in hair samples from forensic scenarios. Further studies will focus on the analysis of a higher number of samples and correlate the data with information on medication intake when available. **Acknowledgments:** The authors would like to acknowledge the financial support from *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES).



FISIOLOGIA E PATOGENESE



Demographic characterization of patients with idiopathic pulmonary fibrosis submitted to lung transplantation in pandemic times

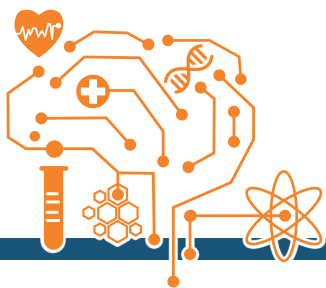
SILVEIRA, Andresa Thomé¹; FLÔR, Juliane da Silva¹; MARTINS, Isabel Amaral¹; SCHERER, Laura Bastos Otero¹; SILVA, Laura Tibola Marques¹; MACIEL, Lorenzo Santana¹; RIGATTO, Katya¹

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Introduction: Idiopathic pulmonary fibrosis (IPF) is considered a non-infectious chronic disease, whose etiology and physiological mechanism are still not fully understood. This disease can have a slow and progressive course, resulting in a median survival of only three years after diagnosis. Due to its chronic characteristics and therapeutic limitations, IPF is in the group of interstitial lung diseases that cause more significant mortality and respiratory morbidity worldwide. To date, no drug is capable of curing IPF, only reducing the symptoms of the disease, making lung transplantation a therapeutic option. This procedure can promote an improvement in the quality of life and more prolonged survival for these patients. **Objective:** To describe the demographic characteristics of patients diagnosed with IPF undergoing lung transplantation carried out at a referral center for transplantation in Latin America. **Method:** This is a descriptive observational study undergoing from January 2020 to September 2022. The research was approved according to the CAAE opinion: 69947517.2.0000.5345 UFCSPA and CAAE: 69947517.2.3001.5335 ISCMPA and

all participants signed an informed consent form.

Results: The mean age was 58.42 years (± 8.69 years), 75% were male, 58.3% underwent left unilateral transplantation, presenting pre-surgical spirometry values, respectively, FEV1 E.99% (± 14.28) and FVCC.22% (± 13.21). According to the Brazilian Pulmonary Function Guidelines classification, the IPF patients undergoing transplantation in this center had a moderate FEV1 indicating that functional loss is always present, being an important marker of poor prognosis. **Conclusion:** Although with a significant reduction in donors and with all the setbacks imposed by the Covid-19 pandemic, some patients had the opportunity to perform lung transplantation to improve their quality of life. Of note, epidemiological studies on this population are still scarce in Brazil, making them extremely important for a better understanding of the disease and the development of treatment. **Acknowledgments:** We are particularly indebted to the Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior and to the volunteers participating in the research.



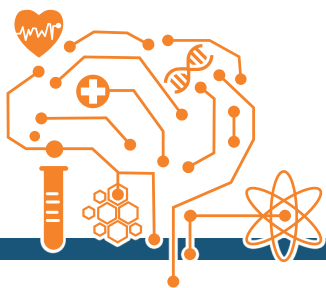
Disease severity and systemic phenotype impair sympathetic nervous system response in patients with COPD

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Background: Cardiac autonomic dysfunction is an important complication of chronic obstructive pulmonary disease (COPD). However, the association between cardiac dysfunction and the severity profile of COPD patients, as well as the concomitant occurrence of COPD phenotypes, is not well characterized. **Objective:** To investigate whether the heart rate variability (HRV) differs between COPD phenotypes and COPD severity, both at rest and after sympathetic stimulation. **Methods:** This is a cross-sectional study assessing 46 COPD patients followed at a University Hospital's outpatient respiratory clinic. Electrocardiography was used to record HRV signals both at rest and after performing a maximum voluntary ventilation (MVV) maneuver to stimulate sympathetic participation. Analysis of covariance was applied to examine the influence of COPD severity and three COPD phenotypes (asthma-COPD overlap syndrome (ACOS), chronic bronchitis, and systemic phenotype) on HRV results at rest. The Wilcoxon signed-rank test assessed the effect of the MVV sympathetic stimulation within each group. **Results:** At rest, patients with severe or very severe COPD presented an HRV power that was higher in the high-frequency band both in absolute value (HFa, $p = 0.046$) and normalized units (HFnu, $p = 0.037$) than patients with mild or moderate COPD. The total HRV ($p = 0.025$), the high-frequency band (HFa, $p = 0.002$), and the low-frequency band (LFa,

$p = 0.031$) were lower in patients with a systemic phenotype compared to patients without it. The presence of ACOS or chronic bronchitis phenotype did not influence HRV at rest. Additionally, patients with severe or very severe disease and those with a systemic phenotype were not able to respond to sympathetic stimulation. **Discussion/Conclusion:** COPD severity and the systemic phenotype were consistently related to impaired cardiac autonomic regulation. At rest, COPD severity was related to decreased sympathetic participation, while the systemic phenotype was related to an overall reduction in HRV. The absence of a modulatory response to sympathetic stimulation using the MVV maneuver suggests possible sympathetic nervous system exhaustion in patients with severe/very severe COPD and those with the systemic phenotype, which could impact the clinical prognosis of these patients and potentially impair cardiovascular outcomes. Additional studies would help refine the implications of sympathetic modulation in the progression of COPD as a systemic disease. **Acknowledgments:** The authors acknowledge the help of Prof. Adalberto Sperb Rubin for providing access to the outpatient clinic at the Irmandade Santa Casa de Misericórdia Hospital in Porto Alegre, Brazil. We are grateful to Prof. Tiago Becker for the spectral analyses of the ECG tracings.



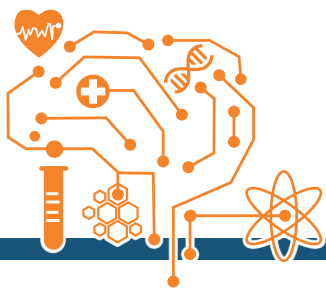
In vitro study on the cytotoxicity of alamandine and its effects on cell proliferation

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Background: Pulmonary Fibrosis (PF) is a chronic fibrosing interstitial disease, its cause unknown related to a progressive worsening of respiratory function, there is a high rate of morbidity and mortality, is a shortage of drugs to treat it. PF is a disease that primarily affects adults between the ages of 60 and 70 years. As it is a multifactorial disease that does not have a defined etiology or pathophysiological mechanisms, the alternatives for effective treatments are still limited. The involvement of the renin-angiotensin system (RAS) with PF is scientifically recognized in the literature. RAS is altered in patients with PF, in whom a significant reduction in plasma alamandine (ALA) was observed. ALA already demonstrates protective physiological actions in several systems and also in the lung, suggesting the possibility of becoming a promising proposal for treatment. **Objective:** this work seach to evaluate the viability of culturing MRC-5 cells exposed to different concentrations of ALA, as well as to observe the effects of ALA administration in cultures of primary fibroblasts isolated from fibrous lungs of rats submitted to a PF model by Bleomycin (BLM), aiming to test a possible therapeutic potential of this peptide. **Methods:** In order to verify the cell viability of MRC5 human fibroblast lines exposed to increasing concentrations of Alamandine (10⁻⁴, 10⁻⁵, 10⁻⁶, 10⁻⁷, 10⁻⁸ and 10⁻⁹ M) in the acute phase (72hours), the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay was performed. Cells were plated and treated in a 96-well plate, cultured and incubated for 3.5hours at 37°C in MTT solution (0.5mg/ml). At the end, Formazan crystals formed by the cleavage of tetrazolium were dissolved with 100µL of DMSO (Dimethylsulfoxide) and quantified at 560nm in the spectrophotometer. For the extraction of lung fibroblasts from rats with FP, these animals were induced to FP by one intratracheal

instillation of 0.2ml of BLM solution at a concentration of 2.5mg/kg. On the 14th day after instillation, euthanasia was performed by an anesthetic overdose of ketamine (200mg/kg - Syntec) and xylazine (30mg/kg - Syntec), intraperitoneally, followed by the collection of lung tissue. The tissue was removed and fragmented with the use of sterile surgical materials and instruments. After fragmentation, the tissue was kept in a sterile Petri dish, containing collection medium composed of saline (0.9% NaCl) buffered with PBS phosphate buffer, supplemented with 2% penicillin/streptomycin. Tissues were serially washed in collection medium, divided into smaller fragments and transferred to 6-well polystyrene plates until adhesion. Subsequently, Dulbecco's modified Eagle's medium (DMEM) was added, supplemented with Fetal Bovine Serum (10%) and 1% penicillin/streptomycin. The plates were kept in a CO₂ incubator (5%) with 95% humidity at 37°C. **Results:** The results of the MTT assay were statistically analyzed using one-way ANOVA. None of the tested concentrations of ALA caused cytotoxicity in the MRC5 cell line after 72hrs of exposure, maintaining its viability. The induction of pulmonary fibrosis was successful, as observed by the macroscopic examination after euthanasia, as well as by the subsequent observation, under light microscopy, of primary cells extracted from the fibrous lung of rats. These fibroblasts, when treated with ALA, showed typical morphology and proliferation capacity, which can serve as a means for evaluating the therapeutic potential of ALA. **Discussion and conclusions:** These findings indicate that ALA has the potential to be an alternative treatment for idiopathic pulmonary fibrosis. **Acknowledgments:** This study was financed in part by the Coordination for the Improvement of Higher Education Personnel – Brazil (CAPES) – Finance Code 001.



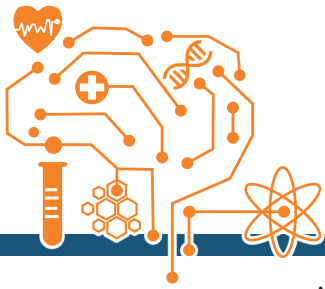
Investigation of the antioxidant capacity of Alamandine

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Background: Pulmonary fibrosis (PF) is a progressive and chronic disease, with high morbidity and mortality worldwide. Therapeutic limitations worsen the prognosis, which has a median survival of only 3 years after diagnosis. Current evidence leaves no doubt about the involvement of oxidative stress in the initiation, progression and worsening of the prognosis of PF. The renin-angiotensin system (RAS) is an important component of the organism's homeostasis and the data to date show that the new components of this system are promising restorers of redox balance. Alamandine (ALA) has been shown to have important antioxidant effects in renal, cardiovascular and fibrosing conditions. However, data on the respiratory system are scarce. **Objective:** Thus, the aim of this study was to evaluate the preventive oxidant potential in the development of experimental PF induced by bleomycin (BLM). **Methods:** Wistar rats were randomly divided into 4 groups: CO (healthy), ALA (healthy and treated with ALA), BLM (fibrotic) and BLM+ALA (fibrotic and treated with ALA). PF was induced by oropharyngeal aspiration (OA) of BLM (2.5mg/kg body weight, Bonar, Ache) dissolved in 0.2 ml of normal saline. On the same day, osmotic minipumps (OM; Alzet 2004) containing 0.2 ml of ALA (Sigma Aldrich, St. Louis, MO, USA) dissolved in 0.2 ml of normal saline were implanted under the skin of the rat. The OM were designed to deliver their contents at 0.25µl/hour (ALA; 50µg/kg or normal saline a day) for 14 days. At the end of the protocol (day 15), all

animals were euthanized by intramuscular anesthetic overdose (240 mg/kg of ketamine and 30 mg/kg of xylazine) and the lungs were collected for analysis of the redox status. The total antioxidant capacity (TAC) of plasma samples was evaluated by the equivalent antioxidant capacity assay of (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Sigma-Aldrich). The total GSH content was analyzed by the 5,5'-dithiobis-[2-nitrobenzoic acid] method, DTNB-GSSG recycling proposed by Griffith. **Results:** We show that rats induced with BLM have a decreased antioxidant defense, indicated by reduced concentrations of GSH and the total antioxidant capacity in the pulmonary tissue compared to the control animals. On the other hand, the animals that received ALA had this capacity restored. For the first time, we found that subcutaneous administration of ALA for 14 days significantly increases the levels of GSH in the lungs of fibrotic rats (P <0.0001 versus BLM group). **Discussion/Conclusion:** Although oxidative stress parameters are not used as markers of disease severity, they are directly involved in fibrotic pathogenesis. Thus, mitigating this imbalance seems to be essential to relieve lung injuries, reduce functional impairments and, consequently, improve the quality of life and the prognosis of these patients. These results are fundamental and indicate other potential pathways of ALA for treatment of pulmonary fibrosis.



Manufacturing 4.0 supporting the provision of PPE and face masks to the frontline and community

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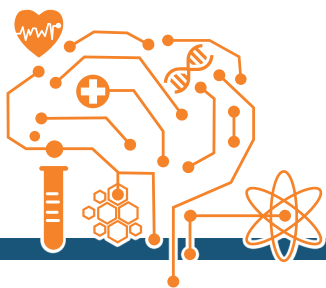
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The high demand for Medical Devices and Personal Protective Equipment (PPE) during the COVID-19 crisis has left millions of healthcare professionals unprotected and occurred at different levels of severity, globally. Developing countries, unfortunately, have been hit hardest by the shortage of medical and PPE to fight COVID-19. Faced with this alarming situation, initiatives adopted collectively or by individuals engaged in digital fabrication have grown, with the aim of mitigating the consequences of the pandemic. However, it is important to note that even though regulatory agencies have relaxed the rules for manufacturing and importing hospital products, regulations exist and must be strictly followed. The Innovation, Prototyping, Creative and Inclusive Education Laboratory (LIPECIN), a makerspace in the Innovation Center of the Federal University of Health Sciences of Porto Alegre and Irmandade Santa Casa de Misericórdia de Porto Alegre, developed strategies for the extenuation of the epidemiological situation that we experienced. Therefore, the objective of this study is to digitally manufacture PPE (face shields), for use in health services, and cotton masks impregnated with zinc oxide (ZnO), for users who do not work in a hospital environment, ensuring the usability and ergonomics of the devices, due to the emergency related to SARS-CoV-2. The Face Shields for use in the local hospital environment were manufactured using two materials: sheets of polyethene terephthalate glycol (PETG) with a thickness of 0.75 mm, for the front visor and forehead strip, and acrylic connectors 5 mm thickness for coupling the visor to the suspension system. PETG plates are readily sterilizable and suitable for use in contact with human skin. A CO₂ laser cutter with a nominal 130 W laser (Katto Laser/1610) was used in the present case. The visors and the suspension system were cut at an average speed of 10 mm/s at 30% of the power. The production of cotton masks also used the laser cutting machine to cut the fabric. The processes of

synthesis and impregnation of ZnO to the textile substrate were based on the work of Azevedo (2016) and Carús (2019) and were later validated through Scanning Microscopy and microbiological tests. The Research Ethics Committee approved both studies of the University (34193820.0.0000.5345 and 46244921.9.0000.5345). The group of LIPECIN volunteers was dedicated to the production and assembly of protective equipment, resulting in 2576 units distributed to workers. Up to now, we have received 38 feedbacks about the ergonomic parameters of the PPE created, through a questionnaire structured with 13 questions about demographics, area of operation, usability, hygiene, and safety. To validate the cotton mask model, 200 units in small, medium and large sizes were manufactured and distributed to the university community. Users received an information leaflet about the correct hygiene of the mask and accepted to receive a form with questions about their experience when using the material. The computed data, so far, point to the need to improve the finishing techniques of the product, as users report discomfort arising from the friction between the straps of the mask and the ears. Our research incorporates knowledge from Industry 4.0, Health Sciences and Materials Engineering, starting with the identification of problems arising from the SARS-COV-2 crisis, the planning of solutions (which involve close collaboration with the health care workers), their implementation (creation of medical artefacts that reduce the effects of the pandemic), their supervision (constant dialogue with the Nursing, Medicine and Work Engineering teams ensuring the possibility to turn prototypes into products in constant improvement) and the evaluation of their effectiveness with potential scalability to the SUS.

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Plasma levels of amino acids in patients with COVID-19 and its association with disease severity

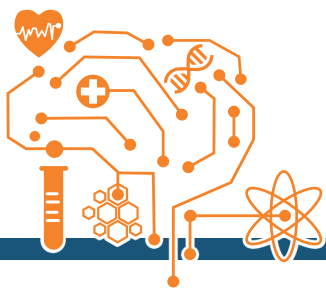
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Introduction: COVID-19 pathology is strongly linked to the unregulated immune response, with emphasis on disturbance in inflammatory mediators, lymphopenia and T cell exhaustion. As a result, such processes related to the disease lead to changes in the availability of different biomolecules, including metabolites that can be used as prognostic markers for COVID-19 severity. In this sense, metabolic disarrangement in COVID-19 is triggered directly by SARS-CoV-2 and indirectly by host response and metabolomic analysis identified some amino acids or their metabolites as candidates for prognostic biomarker. Here we evaluate the plasmatic levels of aspartic acid, glutamic acid, alanine, arginine, asparagine, cysteine, phenylalanine, glycine, glutamine, hydroxyproline, histidine, isoleucine, leucine, lysine, methionine, proline, serine, tyrosine, threonine, tryptophan and valine in controls and patients with COVID-19 (mild, moderate, severe and deceased groups). **Methods:** We recruited 27 healthy individuals (control group) and 171 individuals with COVID-19 for this study (mild COVID-19 (n=28) moderate (n=39), severe COVID-19 (n=50), recovered COVID-19 (n=20) and deceased COVID-19 (n=34), between June 2020 and February 2021. The blood samples were collected at the Emergency Department or at COVID-19 wards including the Intensive Care Unit at Hospital das Clínicas, University of Goiás, Brazil. For individuals who was not hospitalized and recovered were collected at the at Laboratório Prof^a Margarida Dobler Komma, Institute of Tropical Pathology and Public Health, University of Goiás, Goiânia. The research protocol was approved by Ethical Appreciation (CAAE: 30804220.2.0000.5078). LC-MS/MS were used to profile the changes in plasma amino acids of patients with different severities of COVID-19. **Results:** Among all patients, the median age was 56 years for

the control group, 31 years for the mild group, 58 years for moderate group, 57 years for severe group, and 64 years for deceased group. Subjects were 39% (n=11) male and 61% (n=17) female in mild group, 56% (n=22) male and 44% (n=17) female in moderate group, 52% (n=26) male and 48% (n=24) female in severe group, 68% (n=23) male and 32% (n=11) female in deceased group, 33% (n=9) male and 67% (n=18) female in control group. The most common comorbidities in COVID-19 positive patients were hypertension, diabetes mellitus and obesity. The most common symptoms were cough, dyspnea, fever, myalgia, headache, asthenia, diarrhea, anosmia and chest pain. Regarding the analysis of hematological and biochemistry parameters, shows, severe and deceased group presented marked lymphopenia compared to control, mild and moderate groups. On admission, increased plasmatic levels of aspartic acid, glutamic acid and phenylalanine were found in moderate or severe COVID-19 patients compared to controls, whereas cysteine, histidine, lysine, proline, tryptophan levels significantly decreased mainly in severe COVID-19 group. On the other hand, the levels of glutamine, hydroxyproline, isoleucine, leucine, methionine, serine, tyrosine, threonine and valine was not different between control and COVID-19 patients regardless of severity. Moreover, the glutamic acid, alanine, arginine, cysteine, glutamine, lysine, proline, serine, threonine, and tryptophan concentrations were significantly lower in the deceased group compared to the severe COVID-19 group. **Conclusion:** The majority of alterations in amino acids metabolism were found in severe and deceased COVID-19 patients. These changes need to be evaluated along with other parameters currently under analysis to better understand their impact on disease progression and immune profile.

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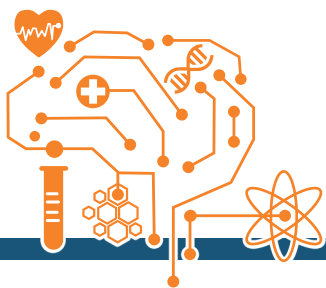
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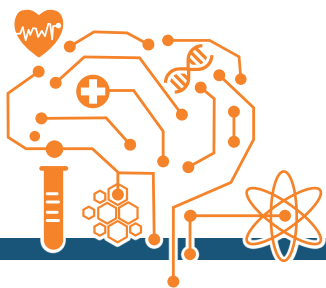
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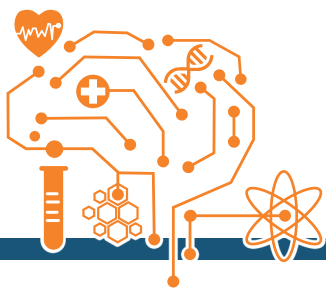
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