

About of supervision of the supe

"Immediate and future challenges to foster One Health"



INTRODUCTORY NOTE

The 6th Congress of the Egas Moniz Center for Interdisciplinary Research was organised under the theme "Immediate and future challenges to foster One Health" and offered researchers and health professionals the opportunity to present and discuss the expected impact of their work in different areas.

These areas included the access to innovative, sustainable, and high quality health care, the use of new tools, technologies, and digital solutions for a healthy society, the promotion of sustainable and circular management of natural resources while tackling pollution, and the security of sustainable food and nutrition systems.

The congress was attended by more than 400 participants, including 9 international invited speakers who delivered their keynote speeches within the congress theme. More than 180 papers were presented at the event, either as posters or oral communications, after a thorough peer-review process.

On behalf of the Organising Committee, we would like to thank all participants for their interest and involvement in this event, the Scientific and Executive Committees for their work and commitment, and the reviewers for their crucial work in ensuring the quality of the papers presented.

Finally, we would like to thank the Egas Moniz School of Health and Science for their continued support of the Congress and all the external partners for their sponsorship.

José Brito, Chairman of the Congress



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ORAL COMMUNICATIONS



Exploring Genetic Variations and Psychological Factors in Alcohol and Drug consumption in a Portuguese Female Sample ⁺

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Drug abuse is a ubiquitous historical phenomenon that largely depends on the cultural and political environment. Portugal de-criminalized the acquisition, and possession of all drugs in 2001, adopting an approach focused on public health. This paradigm shift outcomes are controversial and object of debate with some presenting it as a success and others as a failure. However, there is a lack of empirical data on Portuguese population to measure the drug use trend. The present research aims exploring the intricate interplay between genetic factors and the psychosocial development of individuals with a history of substance abuse. This pilot study examined a sample of 81 female university students with ages ranged from 17 to 40 years, with an average age of 21.04 years (SD = 3.16). For the polymorphism SLC6A4/5-HTTLPR analysis, DNA was obtained from a buccal swab. After DNA extraction 5-HTTLPR genotyping was performed by PCR. The amplification products were analyzed on agarose gel and confirmed by Sanger sequencing. For the psychological variables, three self-report instruments assessed Substance Consumption, Adverse Childhood Experiences (The Adverse Childhood Experiences Questionnaire; ACE), Aggression (The Reactive-Proactive Aggression Questionnaire; RPQ) and Personality (NEO Five-Factor Inventory; NEO-FFI). For alcohol, 39.5% (n=32) of the subjects reported "frequent" consumption, 49.4% (n=40) of the subjects reported "sometimes" consumption and 11.1% (n=9) of the subjects reported never consuming. For cannabis, 9.9% (n=8) reported consuming frequently, 28.4% (n=23) reported consuming "sometimes," and 61.7% (n=50) reported never consuming. For heavier drug consumption, including ecstasy, cocaine, or heroin, 91.4% (n=74) reported never consuming, while 3.7% (n=3) reported consuming "sometimes," and 4.9% (n=4) reported consuming "frequently," accounting for nearly 9% of subjects engaging in hard drug use. The results from the Five Factor Model suggests a positive correlation between Neuroticism and Alcohol Consumption (r = .22, p < .01). The genetic 5-HTTLPR profile of the sample show that for alcohol abuse, 18.1% of the 'consumptions' group had the SS-genotype, while in the 'no consumptions' group, no one with the same genotype was found. For cannabinoids consumption distribution analysis consumers genotype 22.6%, and 22.6% and the 'no consumers' was 32%, and 12%, for LL-, and SS-genotype, respectively. Finally, for ecstasy, cocaine and heroin abuse this trend is even higher. The data suggest a prevalence of cannabis consumption higher than what is reported by the EMCDDA and suggests that carriers of the minor allele of 5-HTTLPR have higher propensity for addiction.

Keywords: genetic variations; psychological factors; consumptions; drugs; alcohol

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Skeletal markers of physiological stress ⁺

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Paleopathology is the study of diseases in the past, including their origins and chrono-geographic distribution. Paleopathogical analysis attempts the reconstruction of health and disease of past human populations, identifying the pathological conditions present in the individual at the time of death from the diagnosis of changes in the skeleton or preserved soft tissues. Structural violence as a consequence of social inequalities may result from unequal access to resources and goods and can lead to a frail health. Human bones and teeth can embody visible manifestations of these difficulted times, as a response to physiological stressors associated with these social factors, and characterizing the skeletal markers of physiological stress. In this presentation, three skeletal markers of physiological stress were examined: cribra orbitalia (CO), porotic hyperostosis (PH), and linear enamel hypoplasias (LEH). We aim to demonstrate the relevance of paleopathogical research, namely the description and interpretation of bone and teeth changes, to a better understanding of the health and living conditions in the past but also in present populations (e.g. epidemiological studies or forensic investigation concerning missing persons). Therefore, skeletal remains housed in the Laboratório de Antropologia Egas Moniz from Egas Moniz School of Health and Science were selected and analyzed, identifying these conditions. Data about individuals' biological profiles were collected. Biological profile and paleopathological conditions were assessed using standard protocols. CO and PH are porotic lesions located in the orbital roof (unilateral or bilateral) and cranial vault, respectively. Their etiology is still not well-understood, been debated by paleopathogist and bioarchaeologist researchers. Many researchers associated these conditions with iron-deficiency anemia. Others refer to scurvy, rickets, infectious diseases, or even traumatic injuries. LEH are deficiencies in enamel matrix composition caused by physiological impairment (disease or nutritional imbalance), macroscopically they are characterized by deficiencies in the amount or thickness of the enamel, manifesting by horizontal grooves on the enamel tooth surface. Results indicated that the samples, where the CO, HP, and LEH cases were identified, with a high prevalence of these markers of physiological stress faced unfavorable living conditions disrupting their growth and physiological balance. On the other hand, can also indicate that despite having gone through unbalanced physiological times, they would possibly have a strong immune system, because they survived. Additionally, the results showed that women had a higher prevalence of physiological stress markers than men, which suggests they may have more efficient immune systems. In conclusion, the skeletal analysis can provide relevant insights about the health and disease, in past and present populations.

Keywords: skeleton; physiological stress; paleopathology; living conditions; health; disease



Sunscreening and photosensitizing therapy in the elderly $^{\rm t}$

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Elderly over 65 years old are generally polymedicated. In this age group, it is important to be aware of the potential for drug-induced photosensitivity. The application of sunscreen is an effective way to prevent sun damage to the skin. A cross-sectional study was conducted aiming to evaluate sunscreen use habits in non-institutionalized elderly people over 65 years of age, living in Portugal and abroad, and the concomitant use of photosensitizing drugs. Elderly taking at least two medicines and living alone or with relatives were invited to participate in the study conducted in May 2023. Participants were requested to show the packages of all medicines they were taking at the time of the interview and a series of demographic and anthropometric data were collected, such as age, gender, nationality and composition of the family household. Additionally, habits of sun protection were assessed through the question "Do you use sunscreen?" Respondents had four options, namely: "never", "rarely", "only in summer" and " regularly". Medicines taken by the elderly were characterized in terms of ATC code, route of administration, prescriber and photosensitizing potential. The potential for drug photosensitivity was assessed through an extensive literature search, including reviews, clinical cases, summary of product characteristics (SmPC), package leaflets (PL), and databases such as Medscape. The association between variables of interest was assessed using the Spearman correlation coefficient or Contingency tables and the chi-square test of independence. All statistical tests were applied at the 5% level of significance, using SPSS 28 (Armonk, NY: IBM Corp). Association between habits of sun protection and nationality was not assessed due to scarcity of the data per cell. A total of 104 seniors (65.4% women and 34.6% men) with an average age of 78.4 years old were included in the study. The majority of the participants live in Portugal (59.6%) followed by 30.8% who live in France, 5.8% in Spain and 3.8% in other countries. All but one of the 104 participants take several photosensitizing drugs, which number is in the range 0 to 13. The quartiles of the number of drugs taken were 2 (Q1), 3 (Q2) and 5 (Q3). The percentage of individuals who do not use sunscreen is undistinguishable from those who use it only in summer (sig. = 0.305), but is higher than those who rarely (sig. = 0.002) or regularly use it (sig. < 0.001). It was concluded that most of the elderly take several photosensitizing drugs, but do not use sunscreen regularly. Although all the therapy was prescribed or advised by health professionals, virtually all the elderly stated they did not receive advice on this matter. To minimize the risk of photodermatoses caused by photosensitizing drugs, it is of utmost importance that the elderly be advised to use sunscreen whenever therapy includes these types of drugs.

Keywords: Drug-induced photosensitivity; Elderly; Sunscreen protection

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Hyposalivation and Xerostomia: Prevalence and Associated Factors in the Elderly[†]

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In the elderly, several chronic diseases and systemic conditions, such as autoimmune diseases, polymedication, and salivary dysfunction, most caused by therapeutic radiation and chemotherapy, are associated with reduced salivary flow. Xerostomia is defined as the perception of dry mouth, whereas hyposalivation is the objective reduction in salivary flow. Though, both conditions can cause signs or symptoms that can affect quality of life. The purpose of this study was to identify and evaluate potential factors associated with xerostomia and/or hyposalivation in a local elderly population. A total of 150 elderly participants, aged 65 years or more, were included in this study, which was carried out at the Egas Moniz Dental Clinic, between December 2021 and May 2022. Sociodemographic data, general health status, the Summated Xerostomia Inventory (SXI- PL) and the Geriatric Oral Health Assessment Index (GOHAI) were obtained through a questionnaire. Hyposalivation was assessed by the rate of stimulated (<0.7 mL/min) and unstimulated (<0.1 mL/min) salivary flow using sialometry. Data were statistically analysed by using descriptive and inferential methodologies (Chi-square and Mann-Whitney tests), through IBM SPSS Statistics v.27.0. A significance level of 5% was used in all inferential analyses. The majority of participants were female (60.0%) and the mean age was 72.9 (\pm 4.0) years. Arterial hypertension was the most common systemic disease (60.7%). More than half were taking medication (86.0%), with antihypertensives being the most common (59.0%). Most participants were taking two or more medications at the same time (67.0%). Almost half of the participants had hyposalivation (48.7%) and 31.3% reported a 'dry mouth perception'. A significant association was found between hyposalivation and xerostomia perception (p < 0.05). Hyposalivation was more common in women (52.2%), those taking 1, 2, 3 or 5+ medications simultaneously (41.9%, 53.3%, 60.6% and 62.5%, respectively), and those taking antidepressants (57.1%). Participants with rheumatoid arthritis and depression were also more likely to have hyposalivation (60.0% and 66.7%, respectively). Although no significant association was found, there was a tendency for individuals with poorer oral healthrelated quality of life to have hyposalivation (57.7%). Xerostomia and hyposalivation, semiologically, allow us to indicate the clinical and subclinical state of the patient. Hyposalivation was a very common condition in this study, which may be explained by the co-existence of some chronic diseases, such as rheumatoid arthritis or depression, and the concomitant use of medication, or by the atrophy of salivary gland tissue associated with ageing. Some medications, such as antidepressants, can cause vasoconstriction in the salivary glands, altering their hydroelectrolyte balance, leading to changes in acinar cell function or glandular tubule structure. Hyposalivation in female participants can be explained by the decrease in progesterone and estrogen due to the menopause, resulting in the salivary glands being unable to absorb these hormones. Hyposalivation is a condition that promotes poorer quality of life in this age group and should be diagnosed and treated to improve the wellbeing of the elderly.

Keywords: Xerostomia; Hyposalivation; Geriatrics



0.5

Adopting an external focus of attention increases torque variability ⁺

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In strength training, especially in rehabilitation when relearning certain movement patterns, it is a common practice to instruct the patient to focus internally, e.g., on a certain muscle. However, recent scientific evidence suggests an external focus of attention, e.g., focusing on the object where the force is applied, as an effective alternative that maximizes task outcome when compared to an internal focus of attention. An external focus promotes a greater number of motor solutions, which seems to derive from an "optimal" amount of variability inherent in the motor system. However, the effect of focus of attention on the variability of force/ torque has not been tested experimentally yet. Therefore, the present study investigated the effect of the type of attentional focus (external vs internal) on the regulation of torque variability and its underlying neurophysiological mechanisms. Fourteen healthy male participants aged 20-29 years performed a 30 second submaximal isometric knee extension task, from which measures of torque variability (sample entropy - the temporal structure; and coefficient of variation - the magnitude of variability) were extracted. Additionally, electromyographic activity of the knee extensor (vastus medialis, vastus lateralis and rectus femoris) and knee flexor (semitendinosus and biceps femoris) muscles was quantified. Furthermore, the cocontraction index between the extensor-flexor pairs was calculated. In the same assessment, the participants performed a maximal isometric knee extension task, where peak torque and rate of torque development were extracted. The maximal tasks were performed three times and the submaximal tasks twice per condition (control, internal, external). The submaximal tasks were executed at 40% of the peak torque obtained in the maximal tasks of the control condition. A oneway repeated measures ANOVA with Tukey's multiple comparison test, or Friedman's ANOVA, were used to test the effect of the condition. In the submaximal tasks, an internal focus (vs an external focus) caused a significant ~5% decrease in sample entropy (i.e., increased regularity), whereas a trend of an increase was found for the coefficient of variation. Additionally, an internal focus (vs an external focus) caused an increase in the muscular activity of vastus medialis and semitendinosus. The co-contraction indices involving semitendinosus showed an increase when adopting an internal instead of an external focus. In the maximal tasks, no differences between conditions were found. Taken together, the increase in regularity and in the coefficient of variation are indicators of a reduced motor control caused by an internal focus when compared to an external focus. The fact that an internal focus (vs an external focus) leads to a general increase in co-contraction and muscular activity, but without affecting maximal force parameters, suggests a lower efficiency of the motor system caused by inter-muscular coordination processes. The present results are of great importance in the context of exercise and health, as changes related to instructions and the task goal seem to impact torque regulation and its neurophysiological mechanisms.

Keywords: motor control; non-linear dynamics; torque complexity; neurophysiology



Profiling oral health status, values, and related quality of life in oral cancer patients: a cross-sectional study ⁺

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Oral cancer is ranked among one of the ten most prevalent cancers worldwide. Patients suffering from oral cancer often do present higher odds towards common dental conditions, lower dental health literacy and related quality of life. Yet such information is not available for the Portuguese scenario. This study aims to report the oral health status of patients with the most common form of oral cancer, oral squamous cell carcinoma (OSCC), medical and sociodemographic data at the Instituto Português de Oncologia de Lisboa Francisco Gentil (IPOLFG). Ultimately, these results will provide a snapshot of the overall condition of these patients and set the tone for importance of oral health among oncology healthcare workers and institutions. This cross-sectional study was developed at the IPOLFG, received approval by the respective Institutional Review Board (Ethics Committee of IPOLFG, ID: 1539) and followed the Declaration of Helsinki of 1975, as revised in 2013. Patients with a first diagnosis of OSCC completed a questionnaire including sociodemographic data, the oral health value scale (OHVS), the short-form oral health impact profile (OHIP-14), Self-Report Measures of Periodontitis. The decay-missing-filled index was recorded through clinical observation. The final sample consisted of 46 patients with a confirmed diagnosis of OSCC, with a predominance of men (34 males vs. 12 females). Participants had an average age of 70.0 years (± 13.2), most of them were retired (65.2%). We found no differences between men and women regarding age (p=0.531), employment status (p=0.114), presence of systemic conditions, smoking habits (p=0.423) or alcohol consumption (p=0.404). The most common systemic disease was hypertension (45.7%). In the OHVS, there was a statistically significant difference between men and women regarding the retention of natural teeth (p=0.021). There were also statistically significant differences between patients' self-perceived presence of periodontitis and functional limitation (p=0.039) and physical pain (p=0.049) from the OHIP-14 questionnaire. In relation to the DMF index, the posterior teeth were lost the most (59.2%). This sample presented considerable high deterioration of oral health other than the diagnosed OSCC. The oral health value and guality of life was severely declined. There is a concerning lack of oral care and health that, consequently, impacts the quality of life of these patients. Therefore, there is an urgent need to implement more oral health programmes and for dentists to play a more active role in the Portuguese public health system.

Keywords: oral cancer; oral squamous cell carcinoma; quality of life; oral health

0.6



Acute Effects of Exercise Modes on Arterial Stiffness and Cardiac Autonomic Function in Healthy Young and Middle-Aged Adults ⁺

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The acute effects of exercise on arterial stiffness (AS), heart rate variability (HRV), and baroreflex sensitivity (BRS) have been extensively studied in laboratory models. Aerobic exercise decreases AS, increases HRV, and improves BRS, while resistance exercise may increase AS and decrease HRV, with uncertain effects on BRS. Combined exercise can have variable effects on these variables depending on intensity, duration, and sequence. However, it remains important to determine if these findings are applicable to ecological exercise settings like group fitness classes, which are increasingly popular for promoting physical activity. The aim of this study was to compare the post-exercise parasympathetic reactivation of the heart, and changes in local and regional indices of AS between different fitness classes, as prescribed for health and commercially available. The intervention involved 24 participants: young (n=12; aged 24.7) and middle-aged adults (n=12; aged 55.1). Participants provided informed consent after an explanation of the study's procedures and aims. The experimental procedures were approved by the ethics committee of Faculdade de Motricidade Humana (12/2019) and aligned with the Declaration of Helsinki. Participants attended 4 intervention sessions of group fitness classes: Bike (BIKE), Pump Power (PP), Global Training (GT), or no exercise (CON), in a randomized order. Each session started with 20 minutes of supine rest, followed by AS assessments, including pulse wave velocity (PWV) and pulse wave analysis of the carotid, brachial, femoral, and distal arteries using applanation tonometry. Carotid AS indices were also analyzed with ultrasound, along with HRV and BRS indices using continuous blood pressure and HR monitoring. After the assessments, participants completed a 45-minute group fitness class. Following exercise, they rested in the supine position for 30 minutes, during which local and regional stiffness, HRV, and BRS were re-evaluated at 10-, 20-, and 30-minute intervals and compared to resting values. Central PWV [F (3, 326)=2.87, p<0.01, n2=0.03) increased immediately after group fitness classes in young and middle-aged adults. Peripheral PWV did not change, but a decrease in peripheral systolic and diastolic blood pressure was observed in both age groups. Local PWV-B was higher in middle-aged adults than in the control condition [F (3, 326) = 3.94, p = 0.09, $\eta 2 = 0.03$)], but no changes in PWV- β were observed after PP in young or middle-aged adults. HRV significantly reduced 10 minutes after the group fitness classes in both age groups for Ln-RMSSD [F (9, 320)=8.14, p<0.01, n2=0.19], Ln-SDNN [F (9, 320)=8.4, p<0.01, n2=0.19], and Ln-HF (F (9, 320)=7.5, p < 0.01, η2=0.17). BRS [F (9, 320)=4.5, p<0.01, η2=0.11)], decreased immediately after all group fitness classes. In conclusion, post-exercise responses were dependent on age, anatomical segments, and time measurements, but not modality. These findings suggest that evidence from laboratory settings on the acute effects of exercise on AS, blood pressure, and the autonomic nervous system cannot be fully transposed to ecological settings.

Keywords: acute exercise; arterial stiffness; cardiovagal modulation; heart rate variability; baroreflex sensitivity; aerobic exercise; resistance exercise; exercise combinations; young adults; middle-aged adults

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0.7



Risk factors prevalence in handball athletes with and without overuse injury history ⁺

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Handball is a demanding team sport with a high risk of injuries, particularly indirect or noncontact injuries. Overuse injuries, resulting from cumulative energy transfers, are common in handball and can lead to reduced training volume, pain, and decreased performance. Deficits in joint range of motion (ROM), external/internal rotator strength ratio, and scapular dyskinesis have been identified as modifiable intrinsic risk factors. This study aims to compare the prevalence of risk factors as rotation range of motion, shoulder external/internal rotator strength ratio, and scapular dyskinesis and their association with sociodemographic characteristics in handball athletes with and without a history of overuse injury. A cross-sectional study design was employed, involving registered male handball athletes from senior and under-20 teams in the 2nd division from Lisbon and Setubal districts. The athletes with a history of dominant shoulder overuse injury in the last 12 months were assigned to the injury group, while exclusion criteria were being underage, having cognitive impairments, recent orthopedic shoulder surgery, or traumatic shoulder injury. Three measurements with a 30 second rest were taken using the Kforce-Link® pull dynamometer to assess the external/internal rotator force, a digital inclinometer, through the Clinometer® smartphone application, to evaluate shoulder ROM, and a measuring tape to assess scapular displacement through the Lateral Scapular Slide Test. A total of 59 participants integrated the study. Healthy group had 39 athletes with 22,8 \pm 5,3 years with a body mass index (BMI) of 26,9 \pm 4,5 kg/m² and unhealthy group had 20 athletes 24,0 \pm 7,0 years and BMI of 25,1 \pm 3,7 kg/m². No significant differences in most variables between groups, except for scapular displacement in standing position, at 90° shoulder abduction and 45° horizontal abduction, with maximum internal rotation of the shoulder and external rotation ROM. Risk factors prevalence had rates under 50% in both groups except for scapular dyskinesis. Functional adaptations in handball may lead to a shift in ROM, with a reduction in internal rotation and an increase in external rotation. Such alterations can contribute to selective muscle inhibition and atrophy, and a higher risk of overuse injuries. The study emphasized the importance of identifying changes in ROM, strength of rotational movements and scapular position to identify athletes at risk of injury. The findings suggested that an additional hour of training reduced the probability of having an internal rotation deficit, and each additional year of training decreased the risk of having a higher external rotator strength when compared to internal rotation measurements. Scapular dyskinesis with higher differences were found close to the shooting position in injury group athletes on the dominant side, which could present as a contributing factor to their injury history. Study limitations included potential measurement errors and the cross-sectional design, which prevented establishing causality. Future studies should adopt a longitudinal approach and improve measurement strategies to further investigate these findings. In conclusion, assessing ROM, scapular dyskinesis, and external/internal rotator strength ratio in handball athletes, regardless of injury history, is crucial for developing injury prevention programs and targeted rehabilitation. Both healthy athletes and those with a history of injury exhibit high rates of identified risk factors.

Keywords: handball; overhead; shoulder; overuse injury; risk factors; prevention.

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Narratives of hope – the temporal dimension in the ontological manipulation of the human embryo[†]

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The human embryo in vitro is a liminal entity that gives rise to plural conceptualisations by beneficiaries of assisted reproductive technologies (ART). Namely, within this ontological manipulation, embryos can be perceived according to distinct equivalence classes/categories: biological material, genetic heritage, offspring, a gift for other couples or to science, potential person, child, etc. However, the meaning-making processes do not vary only between beneficiaries. These conceptions can also change throughout the therapeutic trajectory of each beneficiary. Particularly, hope emerges as a central vector of ontological manipulation, as a lens that guides the actor's perspective towards a particular phenomenon situated in the future. Thus, different narratives of hope that patients build concerning the outcome of the treatment (i.e., achieving pregnancy) can have repercussions at the ontological level, particularly in terms of oscillations in the moral status attributed to the human embryo. This presentation is based on a research project named ETHICHO-Ethical-Ontological Choreographies, developed in Portugal. It encompassed interviews with professionals (medical doctors, clinical embryologists, nurses, and psychologists) and beneficiaries/patients in Portuguese ART clinics/units. The presentation will privilege the thematic analysis of the empirical material gathered from 69 semi-directive interviews with ART beneficiaries, exploring how narratives of hope performed by these patients work as a driving force of ontological manipulation of the embryo. Namely, the respondents' discourses show that the variations in the narratives of hope can take different forms: (a) oscillations between concrete and vague plans, (b) tensions/ambivalences between projecting and dimming the future and (c) combinations between expectation and desire when engaging in the therapeutic plan. These narratives of hope will, in turn, have repercussions at an ontological level, i.e., on the meaning-making processes around the human embryos generated during the ART treatments. That is the case of delaying or withdrawing emotional attachment. Thus, we can identify a double pluralism in the ontological constructions around this entity, in the sense that the ontological markers punctuating the clinical path of the beneficiaries can change according to different circumstances and vicissitudes of their therapeutic trajectory.

Keywords: human embryo; moral status; meaning-making processes; ontological manipulation; narratives of hope



0.10

New compound combining an integrase targeting aptamer and an siRNA targeting the TAR/poly A region of HIV-1 potently suppresses HIV-1 replication[†]

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RNA interference (RNAi) has broad therapeutic potential by silencing gene expression. siRNA, a small synthetic dsRNA sequence, is used to trigger this silencing mechanism. siRNA has been chemically conjugated to bioactive molecules such as lipids, peptides, antibodies, dendrimers and aptamers to improve their pharmacokinetic properties and increase target specificity. Aptamers, small synthetic oligonucleotides with high affinity for biological targets, have been used as targeted delivery systems for siRNA in HIV therapy. The aim of this study was to develop and evaluate the activity of a new aptamer-based siRNA delivery system for HIV gene therapy. The new compound, named Apsi510, was obtained by chemically conjugating an anti-HIV integrase aptamer and a siRNA sequence targeting the HIV-1 TAR/poly A regions to a dendron [2-((4-(2,5-dioxo-2,5-dihydro-1Hpyrrol-1-yl)phenyl)amino)acetaldehyde]. The siRNA was attached to the dendron via an acidtriggered (acid-labile imine) releasing linker allowing effective release in the cell. The activity of Apsi510 against HIV-1 was evaluated in two experimental systems using the subtype B reference isolate HIV-1 NL4.3 and NL4-3.Luc.R-E- in HeLa CD4+ cells and TZM-bl cells, respectively. The results showed that Apsi510 inhibited >95% of HIV-1 replication at 50 nM and that the antiviral activity was dose dependent. siRNA alone and Apsi510 inhibited HIV-1 replication to a similar extent in both experimental systems, indicating efficient intracellular release of the siRNA molecule by Apsi510. The strongest inhibition of HIV-1 replication was obtained by Apsi510 compared to the aptamer or siRNA alone, indicating an additive effect. In conclusion, Apsi510 displays a potent activity against HIV-1 and could be a promising drug candidate for HIV therapy and prevention.

Keywords: Aptamer; siRNA; Apsi510 compound; HIV; antiviral activity



0.11

Characterization of novel fascin inhibitors for blocking invasion and metastasis in colorectal cancer [†]

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Serrated adenocarcinoma (SAC) is a histological subtype of colorectal cancer recognized by the World Health Organization (WHO) and represents about 9% of colorectal carcinomas. Compared with conventional carcinoma (CC), SAC has a worse prognosis and survival. These features are the consequence that SAC induces a weak immune response, in parallel with an aggressive invasive behavior. Furthermore, SAC does not respond efficiently to targeted therapies due to the high KRAS/BRAF frequency mutations. However, possible molecular oncotargets have recently been identified, thus favoring the search for new specific treatments for SAC. In this line, the fascin protein is over-expressed in SAC and arises as a novel drug target. Fascin is a key protein in the rearrangement of the actin cytoskeleton since it participates in the filopodia and lamellipodia formation that are necessary for the cell movement. Due to the fundamental role of fascin in the actin bundles formation, this protein becomes a target for blocking the tumoral invasion and migration, specially of aggressive cancers such as SAC and breast cancer. This study aims to search for new fascin inhibitors in colorectal cancer through pharmacological screening. Subsequently, in vitro assays were performed to evaluate the effects of these inhibitors on the viability (MTT assay), migration (scratch assay) and invasion (matrigel invasion) in colorectal cancer cell lines with different levels of fascin expression (HCT-116 high expression and DLD-1 low expression). There are no immortalized serrated colorectal carcinoma cells. At the same time, the anti-metastatic capacities of the inhibitors were evaluated in a zebrafish model. In silico screening of 9591 compounds, including 2037 approved by the Food and Drug Administration (FDA) was performed, and selected compounds were analyzed for their fascin binding affinity. As a result of these studies, we have identified several compounds as potential fascin inhibitors, including the antidepressant imipramine (IMIP), the antiviral drug raltegravir (RAL) and a non-FDA drug with antimitotic properties, monastrol (MON). Cells showed a migration 41.9±2% (IMIP); 84.3±2% (RAL); 70±2% (MON) and 44.5±4% (MGS) compared to control group. Recent studies have also introduced the use of approved drugs, such as those developed to treat diabetes (Metformin) or thrombosis (acetyl salicylic acid), as coadjutant therapy or even replace more cytotoxic drugs. The anti-metastatic effect has also been observed in an in vivo zebrafish model: % invasion in larvae was 39±2% (IMIP); 29±2% (RAL); 35±2% (MON) and 38±2% (MGS) compared to control group. High Throughput Screening (HTS) plays an important role in translational research. We have found new anti-migratory and anti-invasive properties of IMIP, RAL and MON in colorectal cancer cells. In addition, there is a dose-dependent effect of these compounds that has been demonstrated using a zebrafish model of tumor invasion and metastasis. These studies highlight the real utility of drug repurposing for clinical research. Finally, these findings could be of therapeutic interest for the treatment of metastatic tumor cells such as those found in SAC.

Keywords: colorectal cancer, fascin, inhibitors, imipramine, raltegravir, monastrol, repurposing, migration, invasion, metastasis



Microfluidic fabrication of cell-loaded delivery systems for regenerative medicine applications ⁺

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Chronic wounds are a public health problem of the utmost importance due to the increasing economic and political concerns, both in terms of the financial burdens on healthcare units and the impact it has in patients' quality of life. In a 2018 retrospective analysis of Medicare, chronic and nonhealing wounds have shown to affect approximately 8.2 million Medicare beneficiaries, with cost projections for all wounds ranging from \$28.1 to \$96.8 billion. This includes the costs associated with infection management, with surgical wounds and diabetic ulcers being the most expensive to treat. Therefore, appropriate and effective treatments are emerging in regenerative medicine for optimal healing. Cellular therapies have been researched for several decades, and stem cell therapy has shown efficacy and safety in preclinical and clinical studies. However, a major challenge in stem cell therapy is the delivery route since it directly affects the efficiency of cells regenerative effect due to difficult tissue targeting, high shear stress, and low cell survival. To address this challenge, cellloaded delivery systems such as alginate microparticles have shown to support cell viability and functions, while also protecting cells from the environmental stress. The aim of this work is to develop a microfluidic platform for the production of mesenchymal stem cells and insulin-loaded microparticles. The optimization of the microparticle formulation, the study of cells viability, insulin stability and activity are crucial steps to be addressed in this project. The microfluidic platform (Figure 1) is expected to perform sequential operations for the preparation of precipitated and purified microparticles containing the cells and insulin. Once co-encapsulation is optimized to achieve a final cell density of 3×10⁶ cells/mL bead, the subsequent tests will include testing insulin conformational stability, measuring cell viability, conducting cell and insulin release studies, and performing morphological analysis of the microparticles. To date, fabricated microchannels' depth and width measurements are associated to an error of 5.8% and 3.5%, respectively, relative to the designed channels dimensions. Spherical-shaped and stable unloaded microparticles were obtained at different lipidic and aqueous flows (e.g., I) $Q_{oil} = 10 \ \mu L/min$ and $Q_{alginate} = 2 \ \mu L/min$, II) $Q_{oil} = 30 \ \mu L/min$ and $Q_{alginate} = 15 \ \mu L/min$ in the fabricated device. In which condition II allowed better throughput, maintaining the shape and stability of microbeads). However, improvements are required for downstream processing steps. Cell and insulin loaded microparticles are also expected to be spherical-shaped and stable, with minimal loss of insulin stability and cell viability. The coencapsulation efficiency is expected to be higher than 80%. For cell and insulin release studies, depolymerization of alginate microbeads was performed by incubation at 37°C for 1 hour using a concentration of 1 U/mL alginate lyase. After microfluidic platform design and fabrication, microchannels' depth and width measurements suggest that device fabrication by micromilling is effective, but it can be improved. Cell and insulin-loaded microparticle formulation should be further studied to improve and to support cell viability and functions, with the ultimate goal of assisting wound healing and alleviating the burden on healthcare systems.





Figure 1. (a) Microfluidic T-junction device (main plate); (b) 3D microfluidic system. Not to scale.

Keywords: chronic wound healing, regenerative medicine, microparticles, mesenchymal stem cells, microfluidics

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Intra-oral halitosis in periodontitis: the role of tongue coating – A cross-sectional study †

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Halitosis is an unpleasant breath odor that interferes with self-confidence and with people's professional and social life. The aim of this cross-sectional study was to evaluate the impact of tongue coating on intra-oral halitosis in patients with periodontitis. Consecutive patients were assessed for periodontitis, tongue coating, and halitosis. One calibrated examiner performed a full-mouth periodontal examination. Periodontitis was defined according to the AAP/EFP 2018 consensus. Tongue coating was assessed using the Winkel Tongue Coating Index (WTCI). Data were analyzed by descriptive and inferential methodologies. From a total of 71 participants, 51 were evaluated, regarding halitosis status, by VSC counting. From those, 37.3% were diagnosed as exhibiting halitosis (VSC > 80 ppb). WTCI score was found to be positively and significantly correlated with VSC values (rho= 0.473, p<0.001). WTCI may be associated with levels of volatile sulfur compounds (VSCs), when other causes of extra-oral halitosis are excluded. Further intervention studies are mandatory to confirm this association. It becomes important to introduce tongue scraping in oral hygiene instructions.

Keywords: Halitosis; Tongue coating; Periodontitis; Periodontal medicine; Volatile sulfurous compounds.

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0.14

Antimicrobial Resistance under One Health approach ⁺

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Antimicrobial resistance (AMR) is a critical public health threat that affects human, environmental, and animal health. Among global problems, AMR is the one that best illustrates the One Health approach. AMR is linked to each of these three components due to the irresponsible and excessive use of antimicrobials in various sectors (agriculture, cattle raising, and human medicine). Under the pressure of antimicrobial selection, bacteria acquire resistance genes and mobile genetic elements that can spread to other bacteria of the same or different genus. When bacteria acquire resistance to antimicrobials, they also acquire a greater ability to proliferate in animals, humans, and the natural world. Mismanagement of antimicrobials, inadequate infection control, contaminants in the environment, and migration of people and animals infected with resistant bacteria facilitate the spread of resistance. The aim of the study is to provide a molecular characterization of the epidemiology and resistance patterns of multidrug resistant Klebsiella pneumoniae strains under One Health perspective in Portugal. An original retrospective experimental study was performed. Between June 2019 and November 2021, a total of 130 K. pneumoniae strains were collected from three hospitals in Portugal from clinical and environmental settings. Antimicrobial susceptibility testing, by disk diffusion test, and a polymerase chain reaction (PCR) screening for the presence of carbapenemase genes was conducted. The disk diffusion test results showed high resistance rates $(\geq 60\%)$ to amoxicillin-clavulanic acid (98.5%), ertapenem (96.2%), ceftazidime (90.8%), aztreonam (90%), cefotaxime (89.2%), gentamicin (82.3%), cefoxitin (81.5%), imipenem (78.5%), ciprofloxacin (72.3%) and meropenem (68.5%). KPC-3 (60.4%) and OXA-181 (24.3%) were the most predominant carbapenemase genes found, followed by the co-presence of KPC-3+OXA-181 (4.5%), NDM-1 (4.5%), GES-5 (0.9%) and IMP-4 (0.9%). Additionally, the co-production of CTX-M-15 enzyme with carbapenemase genes was present in 37.8% (42/111). The most frequent clones were ST13 (26.9%), ST17 (23.1%), ST147 (14.6%) and ST307 (8.5%). Regarding virulence genes, 86.9% carried versiniabactin genes, while 25.4% carried colibactin genes. Furthermore, 23 different capsular locus types and 8 different antigen locus types were detected. Environmental strains identified at hospital environment have showed similar pattern compared with clinical ones, being KPC-3 producers. Herein, we report a multicentre study of multidrug-resistant K. pneumoniae strains characterizing the molecular epidemiology of carbapenem-resistant strains in Portugal. The most predominant carbapenemase produced were blaKPC-3 and blaOXA-181, with the co-occurrence of



both carbapenemase genes being also found. The concomitant presence of these traits in high-risk clones in hospital and environment is very concerning and warrants further attention. Moreover, infection control measures as well as periodic environmental screenings are crucial for controlling and preventing the dissemination of highly pathogenic strains in diverse settings.

Keywords: antimicrobial resistance; antimicrobial stewardship; One Health; prevention and infection control

Abstract

0.15

Performance of ID NOW Influenza A&B 2 ⁺

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ID NOW™ INFLUENZA A&B 2 is a point-of-care assay for rapid molecular diagnosis of Influenza A and B. This system uses isothermal nucleic acid amplification technology, using primers and fluorescent specific probes for amplification of RNA targets without the need for a thermal cy-cler. The present study aims to evaluate the performance of ID NOW™ INFLUENZA A&B 2 compared to a reference RT-PCR. A total of 67 Influenza A/B nasopharyngeal swabs tested by standard RT-PCR (reference method Allplex[™] RP1, Seegene) for diagnostic purposes were also tested with the ID NOW assay, within 24 hours of reception at the laboratory. Of the 17 positive Influenza A and 5 positive Influenza B, 15 (88%) and 5 (100%), respectively, were also detected by the ID NOW assay (overall positive agreement of 95.2%; 95% CI 80.7-99.7). None of the negative Influenza B samples on RT-PCR provide a false-negative result with ID-NOW. However, for Influenza A, 2 false-negatives and 2 false-positives were obtained, with a negative agreement of 96.0% (95% CI: 67.9-97.9) for Influenza type A, 100% for type B and 95.7% (95% CI: 87.2-99.3) for both. The overall agreement was 95.5% and the Kappa value was 86.6%. In addition, no invalid results were obtained in this study. In terms of the estimated diagnostic performance of ID NOW™ INFLUENZA A&B 2, the sensitivity was 88.2% (95% CI: 67.9-97.9) for Influenza type A, 100% for Influenza type B and the specificity was equal or higher than 96.0% (95% CI: 88.7-99.3) for both. In conclusion, the results of this study demonstrate that the ID NOW[™] INFLUENZA A&B 2 assay can be used as a test for rapid and accurate diagnosis of Influenza in clinical practice. ID NOW assay combines high-speed and accuracy. However, it should be emphasized that the point-of care systems cannot replace reference methodology in a transversal way.

Keywords: Influenza A; Influenza B; Point-of-care; Molecular detection

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Dexamethasone&peptide-loaded contact lenses for substitution of intraocular injections ⁺

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Intraocular injections are the current treatment of many pathologies affecting the posterior segment of the eye. However, this form of drug administration is invasive and may have side effects. Eye drops, on the other hand, are associated with a low bioavailability and significant drug loss by lacrimation. Contact lenses (CLs) have been suggested as non-invasive drug reservoirs, which could deliver drugs in a sustained fashion, increase the drug residence time on the cornea and therefore its bioavailability. However, when targeting the posterior segment of the eye, drug released from CLs still encounter the barrier effect of the ocular tissues, which can considerably reduce the efficacy of delivery. The aim of this work is to develop CLs able to simultaneously deliver an anti-inflammatory drug (dexamethasone sodium phosphate, DexSP) and a permeability-enhancer peptide (penetratin), which would act as a drug carrier, to provide a therapeutic effect in the back of the eye and avoid intraocular injections. Ex vivo tests with porcine conjunctivas revealed an increase in the permeability of DexSP in the presence of penetratin. Therefore, hydroxyethyl methacrylate (HEMA) -based hydrogels were prepared and loaded by soaking in a dual solution of peptide and drug. Besides the pristine HEMA-hydrogel (H1), also hydrogels containing acrylic acid (AAc) or aminopropyl methacrylamide (APMA) were prepared. The first (H2) shall present a higher affinity for penetratin, while the second (H3) is expected to interact in a stronger way with DexSP. H4 hydrogel included both AAc and APMA. Sterilization was performed by High Hydrostatic Pressure (HHP), which allows for the use of relatively low temperature (50 °C) thus preserving the drug and peptide from degradation. The hydrogels demonstrated suitable light transmittance and swelling capacity. H3 hydrogel stood out by successfully loading the peptide and drug and being able to release them for at least 7 h, which is compatible with the wearing time of daily CLs (Figure 1A). In vitro cytotoxicity tests were performed with both unloaded and loaded H3 hydrogels on human corneal epithelial cells. Cell viability was higher than 70%, thus confirming that the proposed device is non-cytotoxic according to the ISO 10993-5:2009 standard. HET-CAM assays showed that the hydrogels did not induce hemorrhage, vascular lysis or coagulation and therefore shall be non-irritating for the ocular surface. Finally, in vivo tests (Figure 1B) were performed on rabbits to evaluate the efficacy of the developed device. The ocular distribution of the drug in the ocular tissues after 6 h of CL wearing was studied. It was found an increase (p < 0.05) in the amount of DexSP detected in the cornea and aqueous humor when delivered in the presence of penetratin. Overall, the results suggest that the studied drug+peptide-loaded CLs shall provide a therapeutic effect in the back of the eye being a potential alternative to injections.

Keywords: dendrimer; experimental monomer; experimental dental adhesive; G-IEMA.



Figure 1. *In vitro* release profiles of DexSP and penetratin from the CLs (A); H3 hydrogel was selected as the most promising CL material and was tested *in vivo* (B).

Keywords: contact lens; peptide; dexamethasone; drug release; back of the eye.

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Bond Strength Properties of a Dental Adhesive with a Novel Dendrimer - G-IEMA ⁺

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The study aimed to assess the microtensile bond strength of two experimental adhesive systems to enamel. Two experimental adhesive systems, one with Bis-GMA (EM1) and another with G-IEMA, as a substitute of Bis-GMA (EM2), were developed in our lab. Two commercial adhesives, Futurabond® M+ (VOCO) (FUT) and ScotchbondTM Universal (3M ESPE) (SBU) were chosen as control. Twenty healthy human permanent molars, obtained with informed consent (approved by the Ethics Committee of Egas Moniz School of Health & Science), were cut into halves and randomly divided into eight groups (n=5) according to the application mode (etch-and-rinse or self-etch): FUT_ER, FUT_SE, SBU_ER, SBU_SE, EM1_ER, EM1_SE, EM2_ER, EM2_SE). After each specimen was polished with a 600 SiC grit paper for smear layer simulation, the adhesives were applied according to manufacturer's protocols. The etch-and-rinse method employed Octacid orthophosphoric acid (37%) (Clarben). Schmidt Composite Nanohybrid (MADESPA) was used for resin build-ups. EliparTM DeepCure-S (3M ESPE), a blue LED light-curing device, was used to lightcure the materials at 1200 mW/cm2. After processing, specimens were kept in distilled water for 24 hours at 37 °C. Beams (1mm2±0.2) were obtained through additional sectioning and tested using a universal testing machine with a cross head speed of 0.5mm/min until failure. Data were analyzed using linear mixed models (LMM) with fixed effects, at a significance level of 5%. According to the findings (p=0.033 and p<0.001, respectively), the adhesive and technique had substantial, independent effects on the microtensile bond strength and there was no evidence of an interaction between the method utilized and the adhesive (p=0.985). ScotchbondTM Universal demonstrated considerably greater microtensile bond strength than experimental EM2 regardless of the application method (p=0.031). Despite the limitations of this study, the results lead to the following conclusions: The four universal adhesive systems investigated demonstrated no statistically significant differences in adhesive strength to enamel, regardless of using etch-and-rinse or self-etch adhesive strategies. The experimental universal adhesive system without Bis-GMA exhibited similar adhesive strength to enamel as other universal adhesive systems containing Bis-GMA. The promising performance of the Bis-GMA-free experimental universal adhesive system highlights the need for further investigations, particularly focusing on exploring the potential of the G-IEMA dendrimer as a substitute for Bis-GMA in adhesive system compositions.

Keywords: dendrimer; experimental monomer; experimental dental adhesive; G-IEMA.

Acknowledgments: The authors would like to express their gratitude to VOCO GmbH, Cuxhaven, Germany for providing the Futurabond M+ adhesive used in this study. The authors declare that they have no potential conflicts of interest regarding any of the products utilized in this research.



The experiences of nursing students during childbirth: Preliminary results ⁺

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Throughout the nursing program, students encounter different circumstances, such as childbirth, and respond in different ways to the difficulties of being a nurse. Clinical practice experiences are crucial to the education of future nurses, and it is imperative to describe and reflect on these experiences. We aim to present the preliminary findings of a qualitative study conducted with undergraduate nursing students about their experiences of observing childbirth for the first time. The research presented, qualitative in nature, fits into the fundamental theory that allows the theory to be generated using an inductive approach. As a data collection method, we used the survey and the technique chosen is the individual semi-structured interview. Its content was analysed through content analysis technique. This abstract provides a summary of our initial findings from the first six interviews. The average duration of the interviews was 13 minutes and 15 seconds, and the average age of the participants was 21.5 years. After reading and analysing the content of each interview transcript, we classify students' experiences into 10 categories: feelings (f=36), staff nurses interaction (f=10), confrontation with reality (f=8), satisfaction with care (f=8), differences between types of childbirth (f=7), internship's effect on desire to become a parent (f=6), managing adverse situations (f=6), expectations (f=5), future professionals' projects (f=3), and family presence (f=1). The experiences described by students are very wide and include emotions of happiness, sadness, and feeling overwhelmed. These experiences affect their decisions regarding motherhood and their professional goals. Students also mentioned that women are especially vulnerable in this setting and that what they observe, and experience is quite different from what they anticipated. Students also characterise adverse circumstances that induce feelings of sadness. This study examines the experiences gained by students during clinical practise, focusing on the delivery room practise and maternity care experiences. Based on these preliminary findings, we can comprehend the experiences of nursing students through their own words. These findings will enable us to gain a deeper understanding of student experiences and to adapt our curricula to better prepare students for them. During this phase of a student's training, it is crucial to understand and interpret their expectations, sentiments, and feelings. This research will contribute to a greater understanding of the relational and human skill development of students in the context of childbirth care. Thus, future nurses will be able to provide care in a manner that interprets and values women's emotions and feelings, which are crucial for providing improved childbirth assistance.

Keywords: students, nursing; natural childbirth; education, nursing; clinical competence



Effect of medication review on the quality of life of patients with Alzheimer's disease: a pre-post prospective quasi-experimental study [†]

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Alzheimer's Disease (AD) is a progressive neurodegenerative disease, and the existing treatment is only symptomatic, using drugs with a negative safety profile that have a significant impact on patients' quality of life. In the elderly, the use of these pharmacological classes (cholinesterase inhibitors and N-methyl-D-aspartate receptor antagonists) can be associated with comorbidities' exacerbation, drug interactions, or lead to a prescribing cascade. Medication review has shown to have a positive impact on optimizing medication use, which may be also important in individuals with AD. The main goal of this study was to evaluate the effect of a type 2a medication review on the quality of life of individuals with AD. An uncontrolled quasi-experimental pre-post prospective study was undertaken in a community pharmacy in Carregado. Individuals were invited to participate if they were aged 65 or older and had a diagnosis of AD (inferred by medication as a proxy). The intervention consisted of a review of type 2a medications, which included the pharmacotherapeutic history and a face-to-face interview with the patient. With this information, the review was conducted using both explicit (Beers Criteria and EU-7 PIM list) and implicit (PCNE DRP V9.1 classification) criteria. Subsequently, the review was conducted using explicit (Beers Criteria and EU-7 PIM list) and implicit criteria (PCNE DRP classification V9.1). Recommendations for medication optimization were directed at patients, carers or treating physicians. Recommendations for patients were made directly in the community pharmacy, and patient agreement was sought. Recommendations for physicians were sent by letter, requesting response within one month. The primary outcome was assessed using the Quality of Life-Alzheimer's Disease (QOL-AD) questionnaire before and two months after the intervention. Data were analyzed using descriptive statistics (MS Excel v. 2304). We were able to recruit eight individuals with AD, with a mean age of 76±4.59 years, four of which were females (50.0%). Participants presented a mean MMSE of 26,13±2,37 points, and were using a mean of 11,75±3,07 medications. After medication review, a total of 44 drugrelated problems (DRPs) were identified, corresponding to a mean of 5,50±4,33 DRPs per patient. Most DPRs were classified in the safety domain (72.7%; n=32). These PRMs led to a proposal of 27 interventions, with a mean of 3.38±3.00 per patient. Fourteen recommendations were sent to the attending physicians, two of which (14.29%) were accepted and implemented; no response was obtained for the remaining 12 recommendations (85.7%). Thirteen interventions were carried out at the patient level, all of which were accepted and 10 (76.92%) already implemented. The effect of medication review on patients' quality of life can only be assessed at a later stage - with score from second application of the QOL-AD - but considering the limited sample, assessment will be qualitative, considering as baseline average QOL-AD score obtained (33 ± 4.82 points). At the time of this publication, the main findings suggest that full integration of pharmacists in multidisciplinary teams is still not a reality and that stronger investments must be made to facilitate interprofessional communication for the benefit of patients.

Keywords: Alzheimer's disease; dementia; quality of life; medication review; drug related problems

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The effect of treatment with Forsus Fatigue Resistant Device on the position of the third molars [†]

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Orthodontic treatment for growing individuals frequently affects the third molars' eruption path. This effect is especially noticeable during non-extraction dentoalveolar treatment of cases with class II malocclusion. Understanding this concern is essential to avoiding unpredictable side effects such as the third molar's impaction or altering its eruption path. Therefore, this study aimed to evaluate the influence of using the Forsus[™] Fatigue Resistant Device (FFRD) in growing class II malocclusion cases on the position of the maxillary and mandibular third molars. In this retrospective study, the included cohort comprised patients with class II malocclusion (ANB \geq 4) treated with FFRD (n=28; 14 males, 14 females, 13.6-year-old, SD \pm 2.4) were compared with a class I control group undergoing orthodontic treatment (n=27: 12 males and 15 females, 13.2 year-old, SD \pm 1.5). The third molars' inclination, the sagittal, and the vertical positions of the were evaluated on the preand post-treatment panoramic radiographs using the Tavano method. Descriptive statistics and a mixed model repeated measures ANOVA were used to determine the measurement differences between the two time points in each group at a significant level of 5%. The only statistically significant difference between the two groups was in the vertical position of the lower left third molar, which was more proximal to the menton plane by -2.24mm in the Forsus group compared to the control group (P=0.010). However, all the other measurements were similar in both groups (P>0.05). Therefore, this study concludes that orthodontic treatment of class II malocclusion using FFRD device does not seem to influence the eruption path of third molars, and the probability of the eruption of third molars is multifactorial and does not rely only on the orthodontic treatment with FFDR.

Keywords: Forsus, Functional appliance, Mandibular propulsion, Third molar



External cervical resorption – The commonly misdiagnosed, destructive resorption – A pilot study [†]

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External cervical resorption is a relatively rare, often misdiagnosed, destructive form of external resorption. It is characterized by uncontrolled growth of resorptive tissue and consequent loss of mineralized tooth tissue in an apical direction. For this reason, it can affect all areas of enamel, dentin and cementum. The clastic cells are responsible for this process, which can lead to severe deterioration and eventual tooth loss. This pathological resorption is asymptomatic and, nowadays, an aetiological factor has not yet been found. Even so, trauma, periodontal disease or orthodontic treatment may be predisposing factors. Therefore, dentists and students still have difficulties in diagnosing and treating it. For this reason, there are some symptoms which are crucial to diagnose this resorption. Firstly, as the name suggests, it occurs in the cervical part of the tooth, below the epithelial junction but supra-osseous. In general, it is shown as a restricted area, both clinically and radiographically. On radiographs it may appear as a radiolucent well-defined lesion. In addition, in some cases it can be seen as a pinkish coronal discoloration. Nevertheless, for the diagnosis and assessment of a resorptive lesion, the use of cone beam computed tomography (CBCT) is recommended. This helps to diagnose and assess the entry point. For this reason, it may facilitate the selection of an appropriate treatment approach, especially in advanced cases. In this pilot study, a structured questionnaire was administered to one hundred and four people via Google Forms, in May 2023. The following inclusion criteria were only dentists, fourth- or fifth-year dentistry students. Of the one hundred and four responses, one hundred and one were usable. The questionnaire was used to assess the knowledge, perception, and clinical experience towards external cervical resorption. Among the volunteers, 99.0% claimed to be aware of tooth resorptions, although only 45.2% were aware of external cervical resorption. Only those who were aware of this pathology were included for further analysis. Among the interviewees who felt comfortable diagnosing this lesion (45.7%) only 5 people (23.8%) got all the answers correct. Undoubtedly, external cervical resorption is a devastating form of external resorption and there are still difficulties among dentists and students in its diagnosis and treatment. For this reason, it remains crucial to educate either dentists or dentistry students about this type of pathology, so that it can be properly diagnosed and treated, avoiding tooth loss and major complications.

Keywords: external cervical resorption; external resorption; diagnose; treatment.



Digital Compounding in Pharmacies – A Pilot Stability Study [†]

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The design and manufacture of patient-centric medicines is critical to attain unmet medical/clinical needs. The manufacture of medicines on-demand for a particular patient, at the point of care, may be achieved by 3D printing, which allows digital customization of the dose and/or of the design of the dosage form to tailor drug release, improving therapeutic outcomes, medication adherence and patient safety. Solid dosage forms are often 3D-printed by combining hot-melt extrusion (HME, for the production of filaments to feed the printer) and fused deposition modelling (FDM). FDM printers are easy to install in community and hospital pharmacies, allowing decentralization of production, close to the patient, anywhere in the world. The necessary supervision by the pharmacist, adds value to its role in compounding, besides presenting therapeutic advantages to the patient. To fulfil this goal, filaments are expected to be prepared in-house, or by industry, to allow fast compounding when needed. In either case, drug-loaded filaments are required to maintain stability and printability over time. In this work, paracetamol-loaded hydroxypropylcellulose (HPC) filaments were produced by HME from six different formulations; extrudability and printability were aided by the use of a plasticizer and a lubricant. Filaments were characterized in terms of dimensions, mechanical behaviour and drug loading, as well as sensitivity to moisture. For printability, 11% humidity was the ideal storage condition of filaments, up to 6 months. The tablets produced were in agreement with the digital template in terms of dimensions, and complied with uniformity of mass and content. After in-use stability testing for 30 days, tablets maintained identical mass, dimensions, and amount of drug, regardless of the storage conditions, suggesting a good and desirable stability of both the matrix and drug. In vitro dissolution testing of the tablets showed delayed drug release, maintained at the end of the in-use stability test; comparison with the initial corresponding release profile showed similarity between the curves (f2>50). Due to the swelling of HPC, the matrix expanded in the dissolution media, followed by a steady erosion of the polymer, leading to a somewhat complex release behaviour. Nevertheless, kinetic parameters showed a relationship between the amount of polymeric matrix and dissolution rate, indicating that HPC is indeed controlling the release of paracetamol. These preliminary results add to the narrow information available regarding the stability of filaments and FDM 3D-printed tablets, and may be transposed to other drugs with the required adjustments.

Keywords: 3D-printing; digital compounding; fused deposition modelling (FDM); humidity; paracetamol; pharmacies; storage stability; in-use stability; tablet.

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Intra-rater and inter-rater reliability of the Kinvent hand-held dynamometer in young adults ⁺

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Health professionals like physiotherapists, need more options of accessible hand-helddynamometers to perform muscle strength assessments before and after interventions to quantify treatment effectiveness, patient progression and to adjust rehabilitation goals. New dynamometers have been placed in the market, however, there is no evidence for the Kinvent. The aim of this study was to investigate intra and inter-rater reliability of the Kinvent hand-held dynamometer in muscle groups of the upper and lower limbs. For this purpose, this cross-sectional study followed COSMIN quidelines and it was performed in two days of assessment using Kinvent. The first day included three acceptable measurements of the maximum voluntary isometric contraction for each muscle group for the rater 1 (R1) and for the rater 2 (R2). After 48 hours, the procedure was repeated for the Rater 1. The intra-rater reliability was assessed between the two days of assessment for Rater 1 (ICC one-way-random). The inter-rater reliability was determined by comparing the mean of the three measurements of different movements for each participant made in one day, between two raters. (ICC two-way-random/Bland-Altman). The ICC values were classified as moderate (.50–.75), good (.76–.90), and excellent (>.90), with p-value<0,05. A convenience sample of 12 individuals, being 7 women (58,34%), with 21.83 ± 3.16 years, and 22.76 ± 2.03 kg/m², participated in the study. For the upper limbs, Kinvent showed a good intra-rater reliability for shoulder abduction: .850 (.575-.945); wrist flexion: .854 (.586-.955) and extension .874 (.636-.962); to excellent for elbow flexion .951 (.854-.985) and extension .912 (.734-.973). For inter-rater reliability, an excellent ICC (CI) for shoulder flexion .922 (.273-.905) and abduction .917 (.739-.975); elbow flexion .985 (.948-.996), and extension .961 (.872-.989); and wrist extension .929 (.773-.979) and a good for wrist flexion .894 (.675-.968) were found. For the lower limbs, Kinvent showed good intra-rater reliability for hip flexion .796 (.453-.936) and abduction .840 (.552-.951); excellent for knee flexion .939 (.811-.982) and extension .909 (.726-.972). For inter-rater reliability, it showed good results for knee flexion .863 (.594-.959) and extension .876 (.604-.965); excellent for hip flexion .900 (.692-.970) and abduction .916 (.734-.975). Ankle dorsiflexion was moderate for all assessments. A recent study found good to excellent correlation for the isometric lower limb strength and power in a healthy population, particularly for proximal muscle groups. Our results are in concordance with these findings. Health professionals like physiotherapists, need more options of hand-held dynamometers to perform assessments before and after interventions to quantify treatment effectiveness, patient progression and to adjust rehabilitation goals. Some limitations need to be considered such as the small sample used, and the inexistence of an accessible isokinetic dynamometer to compare the results with the gold standard for muscle strength. In conclusion, the Kinvent showed a good to excellent intra and inter-rater reliability for almost all upper and lower limbs movements assessed.

Keywords: muscle strength, muscle strength dynamometer, upper extremity, lower extremity, dynamometry, intra-rater reliability, inter-rater reliability, muscle performance

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Comparison between digital and paper handwriting – A contribution to graphoscopic analysis[†]

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The advance in technology has facilitated the storage and transmission of information, but has also contributed to an increase in security concerns, particularly in terms of authentication of information and documents. Traditional security elements, such as handwriting and signatures, have been adapted to the digital domain with the emergence of electronic signatures. However, in the forensic context, questions remain about the potential differences between traditional and digital writings. The objective of this study is to compare the characteristics of handwriting performed on paper and digital tablets in order to assess their suitability for forensic examinations and the attribution of authorship. By understanding the similarities and differences between traditional and digital signatures, it may be possible to improve authentication methods and ensure the integrity of digital documents. This pilot study involved 10 forensic sciences students aged from 18 to 20. The participants provided handwriting samples, including four anonymous samples for authorship identification (two positive controls and two negative controls) and gave written consent and were assigned random codes for anonymity. The study required participants to write a given text three times, once in uppercase and twice in lowercase, on paper and using a digital support (8th generation iPad® with an 1st gen Apple Pencil). Blue ink pens and white A4 paper were used according to Judicial Police guidelines. Three calibrated experts conducted forensic handwriting analysis in two stages. The first stage compared handwriting characteristics between mediums for eight participants. The second stage involved graphoscopic analysis to determine the graphic identity of four anonymous samples. The comparison of handwriting between pen-and-paper and digital mediums revealed notable differences in the characteristics of ovals, periods, spacing, and letter size. Detailed analysis also highlighted disparities in the initial and terminal strokes. However, similarities were observed in graphic impulses, inclination, commas, punctuation, accentuation, calligraphic case, baseline, speed, and letter shapes. The study successfully assigned authorship to one anonymous sample, while two samples did not match any of the previously analyzed writings. The findings suggest that texts can be compared in both formats, as demonstrated by similarities in 16 parameters. This pilot study demonstrates the viability of comparing handwriting in both mediums and achieving a 75% success rate in evaluating handwriting authorship. However, it is imperative to highlight the importance of a validated methodology to support the comparison between mediums and accurately attribute handwriting authorship.

Keywords: Graphoscopy, Forensic handwriting analysis, Questioned documents.

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Dental Students Digital Competences Evaluation: Preliminary Results of a Cohort Study [†]

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Digital competence is widely known as a relevant factor for all the stakeholders involved in technology-enabled educational activities. The concept of digital competence is supported by essential Information and Communication Technologies (ICT) skills, including the ability to use technology in an integrated way for work/study, to critically evaluate the technologies used and to actively engage in digital culture. This study aimed to characterize and profile the digital competence of dental students at EMSHS, aligned with Cluster 6 of the Horizon Europe Strategic Plan. Cluster 6 focuses on digital technologies, digital transformation in industry, and space technologies for societal and economic benefits. The research specifically focuses on the digital skills and competences of dental students, which is relevant to digital transformation in education and healthcare. By assessing students' digital skills and identifying challenges, this study contributes to the development of strategies to improve digital competence in higher education. This cohort study involved 503 dental students at EMSHS. Ethics approval was obtained from the Egas Moniz Ethics Committee. A 44-item anonymous questionnaire was used to collect data on socio-demographic characteristics, residential context, family background, digital characteristics, and involvement in digital learning during the COVID-19 pandemic. The preliminary results showed that 78.5% of the participants believed that digital health would play a major role in their future clinical practice. Additionally, 51.3% of the students reported using their digital skills/competences on a daily basis, with a preference for class transcriptions (35.4%) and video-based support contents (30.8%). The majority of participants (94.0%) perceived their digital competences as high or intermediate. However, when evaluated using the Digital Competence Framework (DigComp), only 26.6% of the students classified themselves as highly skilled. The results of this study provide valuable insights on students' digital competence, including the discrepancy between perceived and actual competence. This has implications for education, highlighting the importance of promoting self-awareness and training. These findings can help educational institutions develop strategies to improve digital competence and the online learning experience, fostering digitalization and leveraging technology for progress.

Keywords: digital competence; dental students; higher education; online learning; digcomp



Precision of tooth size measurement in digital models acquired by intraoral scanning and by scanning of plaster models versus conventional cast models [†]

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Traditionally, diagnostic measurements were obtained from plaster models with a digital calliper. The literature recognizes manual measurements using callipers as accurate and reliable. In recent years, conventional plaster models have been replaced by digital models that are becoming more common due to the benefits related with storage, retrieval, reproduction, and communication. The aim of this study was to determine and compare the precision of mesio-distal tooth size measurements in plaster models using a digital calliper with measurements obtained with NemoCast software in digital models acquired through intraoral scanning and by scanning plaster models using the iTero Element Plus scanner. The study sample consisted of 10 patients with full permanent dentition randomly selected from the clinical archives of the postgraduate orthodontics program. Each case had available dental study models and intraoral digital scans. Digital models were acquired both through intra-oral scanning and by scanning the plaster models using the iTero Element 5D Plus scanner (Align Technology, San Jose, CA, USA). One examiner (MJ) was trained in using the three different methods, measuring with a digital calliper, scanning the plaster models and measuring of the 3D virtual images with NemoCast software. The measurements from conventional dental casts were obtained with a 145 mm digital calliper (Hammacher, Solingen, Germany) with 0.01 mm accuracy. Descriptive analysis of the mean error of mesio-distal tooth size duplicate measurements was carried out and the reproducibility of measurements was assessed with the intraclass correlation coefficient (ICC). The intra-examiner mean random errors of mesio-distal tooth size ranged from 0.07 to 0.20 mm in the digital calliper group, from 0.07 to 0.29 mm for the Nemocast software/iTero intraoral scanning and from 0.07 to 0.28 mm for the Nemocast software/iTero scanning of plaster models. Intraclass correlation coefficients (ICC) for mesio-distal tooth size measurements ranged from 0.630 to 0.990 for the digital calliper (mean ICC = 0.921), from 0.524 to 0.965 for Nemocast/ iTero intraoral scanning (mean ICC = 0.885), and from 0.281 to 0.971 for Nemocast/ iTero plaster scanning (mean ICC = 0.855). The overall reproducibility of mesiodistal tooth size measurements on digital models was comparable to direct measurements with a calliper on plaster models. Similar reproducibility was found for measurements performed on digital models acquired by scanning plaster models and by intraoral scanning. A trend towards lower reproducibility was found for measurements of posterior maxillary teeth in digital models.

Keywords: Intraoral scanners; Digital models; Model scanning; 3D models; Model analysis; Orthodontics; Precision; Accuracy

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The role of intramuscular coordination mechanisms on the variation of torque complexity with neuromuscular fatigue ⁺

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Variability is inherent within the healthy human movement and is defined as the normal fluctuations that occur in motor performance across multiple repetitions of a task. The temporal structure of such fluctuations is referred to as physiological complexity and is believed to reflect a system's adaptability to environmental challenges. Specifically, torque complexity reflects the adaptability of motor control and has been proposed as an indirect indicator of the functional capacity of the neuromuscular system. While torque complexity has been shown to decrease with ageing, disease and fatigue, its underlying mechanisms are not yet fully understood. Thus, the present study aimed to investigate the neurophysiological mechanisms underlying torque complexity's changes with the onset of neuromuscular fatigue. Eighteen healthy and young adults (age: 24.89 ± 3.72 yrs; height: 1.77 ± 0.08 m; weight: 76.39 \pm 13.40kg; BMI: 24.23 \pm 3.26kg/m²) took part in the present study and visited the laboratory on one occasion. Measures were taken at baseline and immediately after a fatiguing protocol. Three extension and flexion maximum voluntary isometric contractions (MVIC) and two submaximal isometric contractions at 30% MVIC were collected. Torque signals were sampled continuously, and the metrics of variability and complexity were calculated based on submaximal contractions trials. The coefficient of variation (CV) was used to quantify torque variability, while torque complexity was determined through Sample Entropy (SampEn). Motor unitrelated electromyographic parameters from the Vastus Lateralis (VL) and Vastus Medialis (VM) -Average Firing Rate (FR), Motor Unit Action Potential amplitude (MUAP) and the relationship between these two parameters (FR/MUAP) - were also extracted from the submaximal trials. A stepwise multiple linear regression analysis was conducted to examine the contribution of these parameters to explain changes in torque complexity. The multiple linear regression analysis revealed that FR/MUAPsiope, FR/MUAPintercept and torque's CV significantly explained fatigue-induced changes in torque complexity accounting for 80.5% of its variance. Interestingly, changes in torque complexity were mainly attributed to intramuscular coordination processes, as VL FR/MUAP_{slope} and VM FR/MUAP_{intercept} collectively accounted for 68.5% of the changes. An increase in both these parameters shifts the relationship between MUAP and FR upwards and to the right. Briefly, this means that for the same firing rate, greater motor units are recruited (i.e., higher MUAP) as fatigue installs. Although the underlying mechanisms of torque complexity are not yet fully understood and further research is needed, our data shows that its variation is highly dependent on intramuscular coordination mechanisms, specifically the recruitment of new and higher threshold motor units. Nonetheless, the variation of force fluctuations across the entire operating range of the muscle cannot be explained by a single mechanism. Future research should seek to investigate the neural input from descending pathways to the motoneurons and their associated neuromuscular junctions. Such analysis could provide greater insight into the interactions between components of the neuromuscular system that interact to produce complex patterns of force.

Keywords: non-linear dynamics; torque complexity; intramuscular coordination; neuromuscular fatigue; high-density electromyography

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Vitamin K intake and Mediterranean Diet: prelimnary results of a Portuguese populational survey ⁺

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Vitamin K is a liposoluble vitamin that plays a crucial role in blood coagulation, bone metabolism, and vascular health. Naturally occurring Vitamin K includes phylloquinone (vitamin K1) and several forms of menaguinones (MKn or vitamin K2). Vitamin K1 is mostly found in all photosynthetic organisms including green leafy vegetables, herbs, algae, vegetable oils, and vitamin K2 in dairy, cheese, other fermented products and in animal products such as meat. Most diets contain an adequate amount of vitamin K considering the intake recommendation set at 120 µg/day for men and 90 µg/day for women. However, established reference levels are based on requirements for maintenance of normal coagulation, while additional extrahepatic vitamin K functions have been neglected. It is accepted that further research is needed on this topic, especially since the intake estimates of vitamin K are not consistent and are influenced by factors related to agricultural conditions, or to different eating patterns. In this study we present the preliminary results obtained in the frame of the NutriSafe project, that consists of a populational based study aimed to assess vitamin K intake, with special focus in the Algarve population, and analysing its association with the Mediterranean Diet. An electronic survey, Nutrik (https://form.jotform.com/222044277445353), was created using a previously validated, semi-quantitative, food frequency questionnaire, which assesses vitamin K intake in the last 30-days. Participants also answered the 14-item PREDIMED scale, to assess the adherence to the Mediterranean Diet. At the time of this report, we collected 168 valid replies to the NutriK survey. Participants are mostly women (n=130; 77%). Mean vitamin K intake was $292\pm271.5 \ \mu g/day$ (CI95% [251; 333]), with no significant differences (p=0.095) between genders (women: $314\pm295.9 \ \mu g/day$; men: $218\pm141.8 \ \mu g/day$). We identified 12 participants (7%) with vitamin K intake more than 10% below the adequate intake. Low intake was more prevalent in men (n=5; 13%) than in women (n=7; 5%), but differences were not statistically significant (p=0.254). Overall, we found a low adherence (n=21; 13%) to the Mediterranean Diet, similar (p=0.889) in men (n=33; 87%) and in women (n=114; 88%). We found a significant positive correlation between vitamin K intake and PREDIMED score (r=0.428; p<0.001). Participants that adhere to the Mediterranean Diet also have a significantly higher vitamin K intake (464±361.8 μ g/day; p<0.001) than those with poor adherence (268±248 μ g/day). These preliminary data indicate a low prevalence of deficient vitamin K intake, and that the intake of this nutrient can be increased by following a Mediterranean Diet. Future analyses will allow us to further assess adequate intake of vitamin K, and discuss possible associations with anticoagulant therapy and other sociodemographic and lifestyle data that were collected in the NutriK survey.

Keywords: Vitamin K; Mediterranean Diet; NutriK

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0.29

Seaweed superpowers: eco-innovative one health solution in aquaculture to control disease outbreaks [†]

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Aquaculture plays a key role in human nutrition and socio-economic development in many countries and its contribution is expected to increase in the future to fight hunger and malnutrition of a growing world population. As the fastest-growing sector within animal production, aquaculture continues to increase its share of the world's seafood supply. However, despite significant investments in technological advancements to maximize the production and value of farmed seafood, the sustainability of aquaculture is compromised due to the occurrence of disease outbreaks. These outbreaks often result from intensification of production systems (e.g., high stocking densities and extreme stress levels) as well as from the impacts of climate change (e.g., extreme weather events as marine heatwayes). The effects of climate change are of particular concern, as projected abjotic changes can create an environment that favors the proliferation of pathogens while simultaneously weakening the physiological resilience of marine fish. This issue is especially critical in the rearing of early-life stage animals, which are inherently more vulnerable to stress and exhibit higher mortality rates. To manage disease outbreaks, aquaculture producers commonly rely on the use of antibiotics and pesticides, which pose significant environmental and public health risks, such as introducing pollutants into marine ecosystems and contributing to the development of antimicrobial resistance. Alternative environmentally friendly strategies are therefore urgently needed to ensure the sustainable growth of aquaculture. One such strategy that is gaining attention in animal production is the use of functional feeds enriched with natural immunostimulant ingredients (e.g., plants/seaweeds). This approach offers a cost-effective and efficient alternative to vaccines and antibiotics. In this regard, the present study aimed to explore the use of macroalgae as an ecoinnovative adaptation strategy to enhance immunity and antioxidant responses of juvenile farmed fish. To this end, we conducted 30-day feeding trials using juvenile Sparus aurata and Diplodus sargus as biological models, administering experimental aguafeeds with varying percentages of Laminaria digitata or Asparagopsis taxiformis (CTR-0%, 1.5%, 3%, or 6%). Fish were housed in recirculation aquaculture systems under optimal growth conditions. After 30 days, 9 fish from each treatment were sampled and blood/plasma and spleen were collected to perform a multi-biomarker approach combining innate humoral parameters (immunoglobulin M, antiprotease and peroxidase activity) and oxidative stress biomarkers (lipid peroxidation, catalase activity, glutathione Stransferases, superoxide dismutase, and total antioxidant capacity) to determine the immunostimulatory potential of the different functional diets. The results demonstrated that the inclusion of L. digitata and A. taxiformis in the diets positively influenced the immunity and antioxidant responses of farmed marine juvenile fish. Specifically, the inclusion of 1.5% L. digitata led to the highest increase in antioxidant capacity and immune responses in S. aurata, while the inclusion of 6% A. taxiformis yielded the most favorable outcomes for D. sargus. These findings highlight the potential of brown and red macroalgae as promising functional ingredients in aquafeeds for carnivorous marine fish, contributing to improved welfare and resilience to environmental stressors, including those associated with climate change.

Keywords: aquaculture; climate change; fish immune responses; antioxidant responses; functional feeds; macroalgae; juvenile fish.


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Abstract

0.30

Mixtures of PAHs in *in vivo* and *in vitro* models: assessment of CYP-mediated metabolic bioactivation and detoxification mechanisms ⁺

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Polycyclic aromatic hydrocarbons (PAHs) are persistent contaminants that are present ubiquitously in the environment, due to multiple sources. PAHs are resistant to degradation, and as a result, they persist and accumulate in the environment, where they typically appear as complex mixtures of compounds. These mixtures may produce entirely different toxicological effects on organisms since the interactions between compounds may result in unpredictable outcomes compared to exposure to single PAHs. The main goal of this work is to understand the mode of action (MOA) of environmentally relevant PAH mixtures through *in vitro* (primary and immortalized cells) and in vivo models. For this purpose, an assessment of mechanisms related to PAH's CYP-mediated metabolic bioactivation and detoxification was carried out in primary fish hepatocytes, zebrafish hepatocytes (ZFL cells), human hepatocytes (HepG2 cells) and in vivo. Cells were exposed to 3 different PAHs, Benzo[a]pyrene (B[a]P), Phenanthrene (Phe) and Benzo[b]fluoranthene (B[b]F) with different chemical structures and carcinogenic potential, individually and in mixtures with different ratios (1:1, 1:2, 2:1) during 24h. The in vivo assay encompassed the exposure of fish (giltheaded seabreams) for 42 days to B[a]P, Phe and their mixtures. For both bioassays, the activity and expression of CYP1A1 as well as the activity of several enzymes related to glutathione (GSH) was determined. Overall, a generalized increase in CYP1A1 and consequently in genotoxicity was observed in different experimental models, which was more pronounced in the mixtures than in relation to B[a]P. In the case of GSH, the presence of Phe led to a decrease in GSH synthesis induced by B[b]F. In conclusion, mixtures between different PAHs (carcinogenic and non-carcinogenic) seem to induce phase I metabolism as well as genotoxicity, while decreasing GSH levels which is essential for Phase II conjugation and detoxification. These findings demonstrate that environmental quality guidelines may be underestimating the risk of contamination by PAHs since they only consider PAHs in isolation. Thus, it is crucial to make a more realistic risk assessment, which considers PAHs in complex mixtures as well as in environmentally relevant matrices and models.

Keywords: Zebrafish, Primary Hepatocytes, HepG2, CYP1A1, glutathione

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Healthy and sustainable food choices: the development of an integrative tool for selecting alternative proteins ⁺

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The impact exerted by the food production systems in terms of climate change and global warming, exploitation of natural resources and loss of biodiversity, associated with the growth of the human population, is a major current significant concern. Food production and agriculture contribute up to 30% of all greenhouse gas emissions, occupy 40% of available land, and use 70% of available fresh-water; are among the largest drivers of bio-diversity loss, species extinction, and natural resource degradation. Among the major contributors to this impact, the production of proteins of animal origin, such as red meat and dairy, represents a significant burden. Therefore, the need to transform food systems is urgent and should be supported by decisions settled in assessing and integrating simultaneously the overall health and sustainability impacts. "ALTERNATIVA | Alternative protein sources in the European diets - integrating health risk-benefit and sustainability", a European project funded by the European Food Safety Authority (EFSA), aimed to establish a harmonized and holistic methodology, bringing together and combining knowledge in health risk-benefit assessment of foods and sustainability assessment, considering in addition to health, other aspects as the environmental, social and economic impact of the consumption of certain foods. Transparent guidelines and objective criteria were applied to develop this harmonized approach considering multiple impacts and outputs from different disciplines and to communicate the results to the potentially involved stake-holders. The developed protocol identifies the methodological options for data collection and assessment underlying scenarios analysis, as well as options to communicate results to facilitate the decision-making process for stakeholders. For the health impact assessment, the risk-benefit assessment methodology, which integrates the evaluation of beneficial and adverse health effects of foods, food components and diets, was considered and recommended; for the environmental, economic, and social sustainability assessment of agri-food performance, the Sustainability Assessment of Food and Agriculture systems (SAFA) Guidelines proposed by the FAO (Food and Agriculture Organization) of the United Nations, was included. This approach is expressively useful for the agro-industrial sector. It facilitates the decision process regarding health and sustainability, e.g. identifying the best alternatives to produce foods, including those of animal origin and their alternatives, guaranteeing the sustainability and health aspects of these decisions. This methodology developed under ALTERNATIVA constitutes a contribution to minimizing the effects of the current pressures exerted by the food systems, trying to provide safe, economically fair, affordable, nutritionally adequate, and healthy diets having lower impacts on the sustainability of food systems.

Keywords: Health; Sustainability; Risk-benefit assessment; Food systems; Informed decisions

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Abstract

0.32

Bioconversion of catering waste by the Black Soldier Fly (*Hermetia illucens*): a One Health perspective

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The world's population continues to grow, and this is having an impact on people's lives. One of the issues raised is the increase in urban organic wastes (UOWs), which consists mainly of food waste, and is responsible for large amounts of greenhouse gas emissions when it rots in landfills. The most common approaches to dealing with organic waste in the European Union (EU), such as landfilling and incineration/combustion, are not environmentally friendly. As populations grow, not only is climate change worsening, but so are food insecurity issues. Bioconversion of UOWs by insects such as the Black Soldier Fly (BSF) is a sustainable way to address these problems. BSF larvae can feed on organic waste, such as catering waste, and convert it into new valuable products, e.g., larval biomass and insect frass, which can be used as animal feed and fertiliser, respectively. This process falls under the concept of the circular economy, as food waste is reintroduced into the agri-food chain, bringing economic as well as environmental benefits. However, catering waste is not approved as an insect substrate in the EU, as the European Food Safety Authority (EFSA) states that there is a lack of knowledge about its chemical and microbiological risk profile. EFSA is encouraging research into this subject, considering that different types of impacts and challenges are potentially present, and affecting directly or indirectly humans, animals, and environment. One Health is a multi-sectoral approach to balancing and optimising the health of people, animals, and the environment. It is best known for tackling antimicrobial resistance and emerging zoonoses, but it also focuses on improving food safety and security, protecting biodiversity and conserving nature, among other subjects. This study aims to improve the safety of bioconversion of catering waste by the BSF larvae under the One Health concept. To achieve this, there are four specific objectives: i) the nutritional, microbiological and toxicological assessment of catering waste; through bacterial cultures with specific media and chemical and toxicological methods; this includes the development of a guide to the use of catering waste as a substrate for BSF; ii) the production of genome edited BSF to trace physically and genetically the BSF used in each production process (bioconversion or not), establishing fluorescent and traceable BSF through a CRISPR protocol; iii) the nutritional, microbiological and toxicological assessment of larval biomass and frass, understanding if the larvae bioaccumulate contaminants; the larvae and frass will be assessed in the same way as in i); and, iv) the mitigation of risks regarding bioconversion of UOWs by the BSF; there will be a comprehensive evaluation of the results obtained in the previous tasks; this will determine the safest UOWs to use, the critical control points and how to mitigate the risks identified along insect production using UOWs. This study will lead to a better understanding of the safety of insects reared on catering waste as a sustainable treatment for food waste and as a source of protein and nutrients for animals and soils, safeguarding human, animal, and environmental health.

Keywords: black soldier fly; bioconversion; catering waste; one health; circular economy; sustainability

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0.33

Effects of exposure to Tetrabromobisphenol A (TBBPA) on mussels (*Mytilus galloprovincialis***)**⁺

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Tetrabromobisphenol A (TBBPA) is one of the most common flame retardants and is used in several industrial applications (e.g., plastic paints, synthetic textiles and electrical devices) as a substitute for polybrominated diphenyl ethers. TBBPA is considered of high concern from an ecotoxicological point of view as it exhibits acute and chronic toxicity to several terrestrial and aquatic organisms. Although it is rapidly metabolized and eliminated by animals, TBBPA has been detected in several environmental matrices (e.g., air, soil, water and sediment) and therefore poses a risk to ecosystems. Similarly, the consumption of food contaminated with TBBPA, such as seafood, poses a risk to human health. TBBPA has also been shown to be an endocrine disruptor contaminant and to be nephrotoxic, hepatotoxic and immuno-toxic, both in vivo and in vitro. Thus, the aim of this study was to assess the effects of TBBPA in mussels exposed to different concentrations of TBBPA. Another objective is the presence and accumulation of TBBPA in the edible component of the animal, which is consumed by humans, therefore important in terms of public health. The mussel (Mytilus galloprovincialis), a bivalve mollusk was used as a biological model in this study. Mussels (n=32) were exposed to different concentrations of TBBPA (0, 1, 10 and 100 µg L-1) for 28 days. At the end of the exposure period, the animals were sampled, and several biomarkers were analyzed (GST, SOD, CAT, LPO, TAC), protein degradation signaling (Ubiquitins), cellular apoptosis (caspase), and Acetylcholinesterase (AChE) which acts as a chemical messenger to propagate nerve impulses across the neuromuscular junction between a nerve and a muscle. Results: the results showed that significant cellular responses (enzymatic and non-enzymatic biomarkers) to combat oxidative stress and possibly apoptosis were triggered mostly in mussels exposed to 100 µg L-1 TBBPA, activating several antioxidant mechanisms. The correlation analysis between the analyzed biomarkers supported these findings, where a significant positive correlation was found between the total antioxidant capacity (TAC) and the other biomarkers. The exposure to TBBPA in M. galloprovincialis, can lead to oxidative stress and potentially accumulates in the edible part of the animal, which may also suggest that their consumption can have implications to human health.

Keywords: Mytilus galloprovincialis; Tetrabromobisphenol A, toxicity, Biomarkers, food safety

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Optimization of Inhalable mPEG-PLGA Nanoparticles for Antibody Encapsulation as a Platform for Lung Cancer Treatment ⁺

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Lung cancer has a high mortality rate among all common cancers, estimated to be responsible for about 1 in 5 cancer deaths. Conventional therapies are usually administered intravenously with low selectivity for tumour cells, requiring high doses to achieve the therapeutic effect, leading to potential side effects. Therapeutic antibodies are useful in treatment due to their higher specificity and bioactivity, and lower toxicity compared to small molecule drugs. Antibody encapsulation into nanoparticles for pulmonary delivery is a promising strategy, which combines targeted and controlled drug delivery with the ability to protect antibody structure and bioactivity. Thus, the aim of this work was the development of mPEG-PLGA nanoparticles formulated into a dry powder by spray-drying aimed at localized lung cancer treatment. The optimization of nanoparticles followed a Design-of-Experiment (DoE) approach to target the desired features: small particle size and good colloidal stability. Between the evaluated surfactants, PVA and Tween®80, the latter provided better colloidal stability for this nanocarrier. The polymer mass and surfactant concentration were considered as variables with a significant effect on nanoparticles properties, namely in particle size. The optimized nanoparticles were produced with 150 mg mPEG-PLGA and 1% Tween[®]80, presenting the lowest particle size of \approx 300 nm, polydispersity index of \approx 0.200, and zeta potential of \approx -25 mV, considered suitable features for antibody encapsulation. The spray-drying optimization revealed that D-mannitol and L-leucine, used in combination, were the best matrix excipients to obtain microparticles. Their use at concentrations of 2% and 1% (w/v), respectively, allowed an increase in the yield up to $\approx 60\%$ and reduction in powder adhesion to the apparatus walls due to leucine ability as dispersibility enhancer. Further studies will focus on antibody loading, aimed at establishing an inhalable lung cancer therapy. So, spray-dried microencapsulated nanoparticles aimed at antibody delivery are herein reported for the first time.

Keywords: Antibody; Antibody encapsulation; Inhalation; Lung cancer; mPEG-PLGA; Nanoparticle; Spray-drying.

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0.34



The use of Black Soldier Fly larvae (*Hermetia illucens* L.1758) in manure bioremediation ⁺

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The world's population is growing exponentially, and it is estimated that the total number of people will reach 9 billion by 2050. This population growth has led to an increased demand for animal products and the generation of substantial waste, including manure. Consequently, one of the major challenges we face is finding efficient methods for waste reuse and recycling. An environmentally sustainable approach involves utilizing organic waste in the substrate composition for the growth of insect larvae. One such species used for this purpose is the black soldier fly (BSF), Hermetia illucens L. (1758). BSF offers several advantages for industrial production. Firstly, this species is not considered a pest, as its oral apparatus is poorly developed and it lacks a sting, making it incapable of transmitting diseases. Additionally, adult BSF rely solely on their energy reserves and do not interact with waste or fresh organic matter, including animal or human food. Furthermore, the life cycle of BSF is relatively short. Numerous studies with BSF have demonstrated its ability to enhance waste quality in various aspects. These include the reduction of organic matter, pathogenic microbial load, greenhouse gas emissions, and pollutants when compared to conventional composting methods, among other benefits. Using BSF larvae (BSFL) as a waste bioremediation tool can provide a cleaner and more effective process compared to traditional composting, while also yielding highquality larval biomass and nutrient-rich organic fertilizer. The objective of the present study was to evaluate the impact of three different types of manure (chicken, beef, and swine) in the substrate preparation, inoculated with 5-day-old BSFL, on the weight of the larvae at 4, 8, 11, and 14 days after inoculation. For such an experimental procedure consisting of three trials was conducted using three types of substrates: chicken manure (n=10), swine manure (n=10), and bovine manure (n=10). These trials were performed consecutively over time. Each trial included two treatment groups: an experimental group and a control group, with five repetitions (n=5) each. In the experimental group, the larvae were inoculated into a substrate composed of a mixture of manure, Gainesville, and water (except in the trial with swine manure where water was not used). On the other hand, the control group utilized a substrate prepared with Gainesville and water. Additionally, the study examined the centesimal and mineral composition of the larvae at 14 days, as well as the larval biomass conversion rate. The results indicated that larvae reared in chicken and swine manure were significantly heavier compared to the control, with weight increases of 53.9% and 36.36%, respectively. The observed bioconversion rates ranged from 9.2% to 18.8%. Based on these findings, it can be concluded that BSFL can successfully develop in manure, and the substrate with the most favourable outcomes in terms of larvae quality was the mixture containing swine manure. Thus, these results demonstrate the potential of this method as an effective means of effluent treatment.

Keywords: Black soldier fly, Bioremediation, Manure



POSTER COMMUNICATIONS



P1 Staying healthy in a rapidly changing society



Paleoncological insights about acrometastases: a Portuguese post-medieval case study [†]

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Diseases of the past can be assessed through paleopathological analysis. Paleopathology aims to reconstruct the health and disease patterns in past populations, identifying pathological changes presented in the skeleton or preserved soft tissues. This knowledge makes it possible to understand the diachronic evolution of diseases, responses from populations to the diseases, and evolutionary processes of the pathogen agents, as well as the natural history of the diseases, and their chronogeographical dissemination. Therefore, paleopathology is a relevant contributor to modern medical and clinical knowledge. Specifically, the study of cancer in the past is carried out through paleoncology. Scientific areas such as the history of medicine, evolutionary medicine, paleoepidemiology, paleopathology, and bioarchaeology can clarify the etiological, epidemiological, and evolutionary factors of cancer that changed through the times. We aim to describe the skeletal manifestations of metastatic bone disease identified in the skeletonized remains of an adult woman, following the paleoncology and clinical literature to better understand the clinical and living-health conditions in present oncological patients. An adult woman exhumed from a funerary crypt of the Chapel of the Holy Spirit, in Loures, dating from the 16th-19th centuries, exhibits bone changes associated with metastatic bone disease, and displays acrometastases which is an uncommon finding in present oncological patients. The bone analysis was performed macroscopically, microscopically (stereomicroscope), and radiologically. Diagnostics followed the paleoncological standard protocols. The identified lesions are mostly osteolytic, affecting the cortical and trabecular bone. They exhibit an oval or round shape and geographic margin, or a moth-eaten margin as observed on the femora and vertebra bodies. The size ranged between 0.6 mm x 0.5 mm and 25 mm x 10.9 mm. The lesions were identified at the most common anatomical sites affected by metastatic bone disease, except for the acrometastases. Indeed, osteolytic lesions in the left hand and feet are the most relevant finding in this individual. Acrometastases represented a poor prognosis, revealing pervasive tumor dissemination with limited survival. Therapy has increased the survivability of present oncological patients, and inherently the risk for acrometastases. However, they are an uncommon finding nowadays, and seem more frequent in the past. The true clinical prevalence of acrometastases is probably underestimated due to underreporting, disregard concerning its severity, disregard to the extremities in routine screening procedures, and medical imaging limitations. In short, this case study may contribute to new insights into the occurrence of acrometastases in living populations.

Keywords: tumors; metastatic bone disease; paleoncology; skeleton; paleopathology



P.2

Moderators of resistance training effects in healthy young women: A systematic review and metaanalysis [†]

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Resistance training (RT) is a popular and effective exercise modality to improve health- and performance-related parameters across the lifespan. However, substantial evidence indicates that women are under-represented in the RT literature due to bias towards the inclusion of men and exclusion of women in studies, resulting in limited RT recommendations. As a result, the aim of the present study was to systematically review and analyse the effects of resistance-based exercise programs (i.e., interventions including RT in addition, we examined whether these effects were modified by RT prescription characteristics (i.e., number of sessions, weekly volume, and intensity). This review was registered in the International Prospective Register of Systematic Reviews under the identifier CRD42020217986. A systematic search was conducted using CINAHL, Embase, LILACS, PubMed, Scielo, SPORTDiscus and Web of Science databases from inception to May 2022. Eligible randomised controlled trials examined the effects of resistance-based exercise programs on outcomes of interest in healthy young women (\leq 35 years). The exclusion criteria were: a) studies involving women with any acute or chronic condition (e.g., obesity, type II diabetes, cancer, chronic haemodialysis, heart failure); b) studies involving within-subject design; c) studies with interventions lasting less than 4 weeks; iv) not reporting sufficient information or specific outcomes included in this review; and d) studies written in a language other than English, Portuguese or Spanish. The risk of bias was evaluated according to the 2nd version of the Cochrane risk-of-bias tool for randomised trials. Meta-analysis was undertaken with a three-level mixed-effects model. Associations between standardised mean difference (SMD) and potential moderators (number of sessions, weekly volume, and intensity) were tested by meta-regression models. Statistical significance was set at an a level of 0.05. Forty articles and a total of 1,312 healthy young women with a median age of 22.4 years were included. Resistance-based exercise programs resulted in a significant improvement of 0.4 SMD (95%CI: 0.2 to 0.5) in lean mass/muscle hypertrophy and 1.2 SMD (95%CI: 0.9 to 1.5) in muscle strength. A higher number of sessions was associated with changes in lean mass/muscle hypertrophy (P=0.009), while a higher weekly volume was a potential moderator of changes in muscle strength (P=0.053). Body fat percentage (-0.4 SMD, 95%CI: -0.6 to -0.1) and muscle power/rapid force (0.6 SMD, 95%CI: 0.2 to 1.1) were significantly improved. The present study also has some limitations: a) most studies included had a high risk of bias; b) there was considerable variability in the methods used to assess body composition and muscle hypertrophy; c) analyses on whole-body lean mass and muscle hypertrophy were conducted combined. In conclusion, a higher RT volume (at least ~72 weekly sets including both lower and upper body exercises) equivalent to three full-body workout sessions, three sets per exercise for eight exercises was associated with greater improvements in lean mass/muscle hypertrophy, muscle strength and body fat percentage, whereas muscle power/rapid force improvements were observed irrespective of prescription characteristics. These findings may help in designing RT programs for muscle hypertrophy, strength and power, and body fat percentage in healthy women.



Keywords: young women; strength training; muscle hypertrophy; muscle strength; muscle power

Abstract

P.3

Zinc: Essential Nutrient or Harmful Additive ⁺

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Zinc (Zn) is an essential nutrient, found in a variety of foods (e.g., vegetables, grains, shellfish, meat, eqgs, and dairy), fortified foods, and also in food supplements (FS). Zn plays a key role in enzyme reactions, transcriptional and translational control/modulation, signal transduction, cell growth, skin health, and immune functions. Although, excessive Zn intake can lead to negative health effects such as interference with cholesterol metabolism, nausea, vomiting, lethargy, anemia, and dizziness. Overconsumption of Zn can also cause deficiencies in other nutrients, such as copper and iron. European legislation (Directive 2002/46/EC) states that the maximum amounts of minerals present in FS per daily recommended portion, as indicated by the manufacturer, shall be established by considering upper safe levels determined by scientific evidence and the intake of minerals derived from other dietary sources. The European Directive 2008/100/EC defines a Recommended Daily Allowance (RDA) of 10 mg for zinc. This study assessed whether the daily dose of Zn indicated on the FS labels met the RDA defined by the EU Directive. A total of 62 FS labels were screened and randomly chosen based on meeting the inclusion criteria were met: adult oral solid dosage forms containing Zn in their composition, indicated on the label, regardless of its intended use. Our results revealed that the daily dose of Zn labeled in 60% of the FS exceeded the RDA. Approximately 27% indicated a daily Zn dose higher than the upper limit (UL) of 25 mg/day defined by the European Food Safety Authority (EFSA, 2018). In one product, the labeled dose (200 mg/day) was 20 times higher than that of DDR and 8 times higher than that of UL. Mineral and vitamin supplements are highly used worldwide, do not require a prescription, and are easily accessible in pharmacies, health shops, supermarkets and on the internet. Additionally, they are often consumed over long periods without any supervision by a health professional, which may increase the health risks associated with excessive or unnecessary FS consumption. Following Horizon Europe's efforts to promote public health and based on the findings of this study, which revealed that many evaluated FS labels indicated high levels of Zn, it is imperative to address the potential risks that may arise from excessive mineral intake via FS. It is crucial that relevant authorities establish strict rules to ensure the safety of FS available in the market, including high-quality control, appropriate labeling, and dosage instructions, and to promote the responsible use of FS. It is of utmost importance to adopt a precautionary approach to the consumption of FS to inform consumers about the hazards of excessive Zn consumption and the use of FS without medical advice.

Keywords: Zinc, Food Supplements, Labels information, Recommend Daily Allowance, Health risk.

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Selenium-containing food supplements and (un)labeled information ⁺

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The consumption of selenium-containing food supplements (FS) is common among individuals seeking to improve their health and prevent disease. Selenium (Se) is an essential mineral that plays an important role in protecting the body against oxidative stress and in regulating thyroid function. In the context of health promotion, the use of FS can be considered a preventive approach for certain health conditions. However, a balanced and varied diet can provide all necessary nutrients, including Se, without the need for supplementation. The recommended dietary allowance (RDA) is defined as average daily level of intake sufficient to meet the nutrient requirements of nearly all (97% - 98%)healthy people, based on scientific knowledge. If these Se-containing FS are used in high doses or in combination with a Se-rich diet or some medications, they can pose a risk to public health. Excessive Se intake can be deleterious to health and lead to fatigue, hair loss, brittle nails, irritability, joint pain, and neurological problems. Se can also interfere with drug absorption, compromising its bioavailability (e.g., some antibiotics and anti-osteoporosis drugs). FS, by legislation, are intended to supplement the normal diet and that, not being a medicine, and cannot claim prophylactic properties to prevent or cure diseases. As such, FS are not intended to treat nutritional deficiencies or deficiencies; thus, FS should not exceed the RDA value. This study evaluated whether the daily Se dose listed on the FS labels complied with the RDA of 55 µg/day specified in the European Union Directive 2008/100/EC. The labels of 50 FS were examined. Selection criteria included adult oral solid pharmaceutical forms containing Se in their composition, indicated on the label, regardless of the purpose of the FS. Most of the surveyed FS showed high levels of Se in their labels. In 68% of FS, the label dose of Se was significantly higher than the RDA value. A clinical trial performed in over 35000 individuals (SELECT study) concluded that for a Se intake of over 330 μ g/day (130 μ g from diet and 200 µg from FS), the risk of developing toxic effects, such as alopecia and dermatitis, is higher than that in individuals not consuming FS containing this mineral. Our study results identified 40% of FS with labeled Se doses equal to or greater than 200 µg Se/day, including 6% SA with labeled doses of 400 μ g/day, well above the upper limit (UL) value of 255 μ g/day set by EFSA (2022). The labeling of FS does not alert to the risks of excessive Se consumption and the use of FS without medical advice or potential interactions with medications. Considering public health promotion, measures should be taken to monitor the quality and safety of commercially available FS compared with pharmaceutical quality control exigencies. Additionally, it is necessary to strictly regulate the labeling of these products and educate consumers about the responsible and safe use of these products.

Keywords: Selenium, Food Supplements, Label information, Recommend Daily Allowance, Health risk.

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Body composition and nutrition intake in amateur swimmers: an exploratory study ⁺

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Swimming is a very demanding sport since it is performed in an unfamiliar environment, as well as the technical difficulty and very heavy training burden. In addition, it is practiced at an amateur level and from a young age. Therefore, there are specific concerns regarding the nutritional status of these athletes. The purpose of this study was to compare the food intake of amateur swimmers on a local team to the dietary guidelines. This study was conducted at a local team and only included swimmers who had a regular training regimen, swam at least three times per week, and had competed within the previous 12 months. The anthropometric evaluation was done according to ISAK protocol including the 7-site skinfold measurement (subscapular, subscapular, triceps, biceps, suprailiac, abdominal, thigh and calf) using a Innovare CESCORF lipocalibrator, four body circumferences (relaxed arm, waist, thigh and calf) using a SECA 201 inelastic perimeter tape, height (SECA 213 stadiometer) and weight (SECA 635 weight scale). Food intake was evaluated through a three-day food diary. To estimate the energy, macronutrients intake was used the Portuguese Food Composition Database. Once calculated, the value of total macronutrient intake it was converted to gram per body weight kilogram (g/kg) and compared with the nutritional recommendations. Data analysis included descriptive statistical analysis, distribution analysis through Kolmogorov-Smirnov test. All the statistical analysis was done in SPSS v20.0 and the significance level was set at 0.05. The sample consisted of 15 individuals in average 23±3 years old, 7 were female. The mean BMI was 23 ± 2.6 kg/m², and only two people were overweight with a BMI average of 27.1 kg/m². The average sum of female skinfolds was 104±30mm, which was slightly above the recommended but not significantly (p>0.05). Whereas the average sum of male skinfolds was 61 ± 16 mm, which was within the acceptable range (p<0.05). The energy intake average in females was 1920kilocalories per day (kcal/day) and 2350kcal/day in males. In what refers to macronutrients, the average carbohydrate intake was 4g/kg significantly than the recommendations (p<0.05), while protein (1.7g/kg) and fat intake was adequate. Most recreational swimmers do not adhere to energy and carbohydrate recommendations which can be deleterious for their performance.

Keywords: swimming; amateur swimmers; food intake; macronutrients intake; anthropometric



A multicenter study on clinical and nutritional characterization of patients with dementia at the admission to continuing care units - Study Protocol ⁺

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Dementia patients present an increased risk of malnutrition due to factors such as cognitive impairment, decreased appetite, changes in taste and smell, and difficulty with chewing and swallowing. Malnutrition in dementia patients can lead to weight loss, muscle wasting, increased risk of infection, impaired wound healing, and decreased quality of life. Therefore, nutritional screening and assessment are critical for managing dementia patients. Assessing the nutritional status of dementia patients involves evaluating the patient's food intake, dietary habits, physical function, anthropometry, and ability to perform daily activities. Once the nutritional assessment is complete, personalized nutritional interventions can be developed to support the patient's health and wellbeing. These interventions may include dietary modifications, supplements, education, and meal planning and preparation support. By prioritizing nutritional assessment and interventions in the care of dementia patients, healthcare providers can improve patient outcomes and quality of life. With the prevalence of dementia increasing globally, effective management strategies such as nutritional assessment and intervention are becoming increasingly important in providing optimal care for dementia patients. This study will include patients diagnosed with Dementia institutionalized in the various selected Montepio Residences, and the acceptance of participation in the study by a representative/relative of the patient. The exclusion criteria include patients who refuse to participate in the study and/or those who have advanced-stage dementia with comorbidities that prevent the application of the battery of tests or affect the evaluation. The patients with Dementia fulfilling all the inclusion criteria and not presenting any of the exclusion criteria, are evaluated at admission in continuing care units, using the following data collection procedure: Demographic data collection: age, date of diagnosis, gender; Collection of clinical data: comorbidities, and digestive clinical manifestations; Nutritional assessment: a) score on the Mini Nutritional Assessment[®] (MNA[®]); b) Anthropometric measurements: weight, height, body mass index (BMI) waist circumference (WC), arm circumference (AC), calf circumference (CC) and triceps skinfold measurement; Cognitive impairment/dementia screening test: The Mini-Mental State Examination (MMSE) and Global Deterioration Scale (GDS). Statistical analysis will be used by SPSS to compare and correlate the clinical and nutritional status of a Portuguese cohort of institutionalized patients diagnosed with dementia. It is anticipated that this study will include at least 100 dementia patients with a predetermined duration of no longer than 24 months. Given the vulnerability of this population, a higher percentage of malnourished patients is expected. By gaining a better understanding of this population's characteristics, it will be possible to develop optimal nutritional interventions in the future. The findings from this study have the potential to enhance the management and care provided to dementia patients, ultimately improving patient outcomes and quality of life. As the prevalence of dementia continues to rise globally, effective nutritional assessment and intervention strategies are increasingly crucial in delivering optimal care for this vulnerable population.

Keywords: Dementia, Nutritional Status; Malnutrition



Renal calculi in paleopathology: the post-medieval child mummy from Setúbal⁺

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Human paleopathology intended to reconstruct the health and disease patterns in the past by assessing ancient diseases and injuries in human remains such as bones and preserved soft tissues, from archaeological and paleontological sites. Evolution and natural history of diseases, responses from populations to diseases, pathogens evolution, and diseases' geographical spreading can be clarified through paleopathological analysis. It can help researchers to understand the prevalence and distribution of diseases throughout history and shed light on the impact of factors such as diet, living conditions, and social and cultural practices on human health. Thus, significant insights into modern medical knowledge can be gained from paleopathology. We will present the pathological profile of a mummy believed to be an anonymous daughter of the Duke of Coimbra D. Jorge de Lencastre (1481-1550), an illegitimate son of the Portuguese King D. João II (1455-1495), and briefly review the paleopathological literature on renal calculi. The mummy is currently housed in the Convent of Jesus, a former convent of Saint Clare nuns, in Setúbal, and is placed in a small metal coffin with a glass on the top. In the coffin, an inscription indicates her father's name. Although the mummy exhibits an excellent state of preservation, the neurocranium is missing, as it was damaged when the corpse was placed into the coffin. An external examination and a medical imaging observation (CT scan) were performed, following the paleopathological standard protocols. The radiopaque mass identified in the left kidney was virtually reconstructed (3D reconstruction) and analyzed. The biological profile assessment indicated that was a female who was between 1.5-2 years of age. A dense rounded radiopague mass located on the left of the hypochondriac region, occupied intravitam by the upper half of the kidney, was identified. The skin retained its integrity indicating that the observed mass was endogenous and compatible with a nephrological condition. Based on the radiological features and differential diagnosis possibilities, renal calculus is the probable diagnostic. In conclusion, an internal, non-destructive examination of a child's mummy allowed the identification of an important paleopathological finding, a renal calculus.

Keywords: Setúbal; Portugal; paleopathology; mummy; kidney stones; renal calculi



Assessment of nutrition status in rehabilitation elderly patients from an integrated long-term care unit based on two nutritional screening tools: a cross-sectional study [†]

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Malnutrition has a high prevalence among the hospitalized elderly and in development countries the most reported cause are the acute and chronic diseases. Additionally, the higher age is also one risk factor of being at nutrition risk or to became malnourished. Malnutrition can lead not only to increase of mortality and morbidity but also to physical decline. The objective of this study was to assess the nutritional status of elderly patients hospitalized for rehabilitation in an integrated longterm care unit. A cross-sectional observational study was carried out at in an integrated long-term care unit among hospitalized patients aged 65 years or older admitted for rehabilitation. The Nutritional Risk Screening (NRS-2002) and Mini Nutritional Assessment (MNA) were the assessment tools applied for all participants. General characteristics data of the participants were also collected regarding medical condition, pharmacological therapy, food allergies and/or intolerances. Body mass index (BMI) calculation, body weight, brachial perimeter, cubit length, knee circumference and kneeheel height were also performed. The cubit length and knee-heel height were used in order to obtain the calculation of estimative of participant height. The knee-heel height was also used to obtain the calculation of estimative of participant weight. This study included a sample of 29 individuals (15 male; 14 female) with a mean weight of 70.29 ± 17.30 Kg and a mean BMI of 26.46 ± 6.35 Kg/m2. The mean brachial perimeter was 29.22 ± 4.47 cm, the mean cubit length was 25.24 ± 2.19 cm, the mean knee circumference was 34.26 ± 4.50 cm, and the mean knee-heel height was 46.38 ± 3.55 cm. The NRS-2002 results showed 34.5% of the participants with nutritional risk. The MNA results showed that 3.4% of the sample was malnourished (only in female), and a risk of malnutrition of 37.9% in the sample, in which was more prevalent in males (53.3%). Most of the sample (58.6%) showed a normal nutritional status assessed through MNA. This study showed a more prevalence of risk of malnutrition in males elderly in rehabilitation. Nutritional risk assessment should be performed in order to adopted a nutritional intervention to improve quality of life of rehabilitation elderly patients.

Keywords: Malnutrition; MNA; NRS-2002; Rehabilitation; Elderly



Influence of Color Vision Impairments on Shade Matching Among Dental Students ⁺

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The accurate selection of tooth shades is crucial in restorative dentistry, and color-vision deficiencies among dental professionals may affect their ability to match shades accurately. The main aim of this study was to evaluate the prevalence of colour-vision deficits among a sample of Portuguese final year dental students, and to correlate these findings with their accuracy in shade selection using a commercial tooth shade scale. A total of 119 final year dental students from Instituto Universitário Egas Moniz (IUEM, Caparica, Portugal) were recruited. Participants were mainly female (75%) and had an age interval of 22-42 years old, with a mean age of 23. The evaluation comprised two stages: an initial colour-vision screening test, based on the Ishihara test, and a posterior corresponding shade selection test using the VITAPAN classical scale (Vita Zahnfabrik, Germany). The Ishihara test was applied under standardizes lighting conditions and included reading numbers displayed inside colored circles. For the second phase of the study, students were given 3 teeth from the VITAPAN classical scale and had to blind match the tooth shade with a complete VITAPAN scale. Descriptive statistics were employed using SPSS v. 26.0. Out of the studied sample, only males presented colour-vision deficiency (4/119 wrongly identified all discs from the Ishihara test). Regarding the VITAPAN classical scale match, in total, 59.4% of the students gave the correct answer. The male gender obtained 54.8% of the correct correspondences, while females presented 61%, with no difference reported between genders. Students found it easier to correctly matched shades within the following order of hues, C>B>A>D. On average, darker shades were more difficult to be correctly matched than lighter ones. The presence of colour vision deficiencies does not seem to affect the correct identification and choice of tooth shade using a commercial scale. The male gender showed higher prevalence of colour-vision deficiencies. Considering the tooth shade scale, easiness of matching seems dependent upon the hue.

Keywords: color; color-vision deficits; shade selection



P.10

The effect of unadjusted mineral supplementation on bone status of preterm infants: an exploratory post-hoc analysis in a mixed-cohort study of fortified human milk ⁺

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Preterm infants are susceptible to metabolic bone disease (MBD), and they need a balanced energy and protein supply for osteoid synthesis and minerals for osteoid mineralization. Human milk (HM) is insufficient to cover the high nutritional requirements of growing preterm infants, therefore the addition of multi-nutrient fortifiers to HM is necessary. In 'standard fortification', the macronutrient content of HM is estimated, and multi-nutrient fortifier for HM is added to achieve recommended nutrient targets. Alternatively, 'target fortification' encompasses the measurement of HM macronutrient content to guide the addition of modular macronutrient supplements to the fortified HM, in order to more precisely reach these nutrient targets. This study explores differences in the prevalence of MBD in infants born at <33 weeks gestation, according to the amounts of extra energy and macronutrients added to fortified HM. It is a post-hoc retrospective secondary analysis nested in a principal single-center mixed-cohort study that compared the effect on growth of two HM fortification methods based on assumed (Group 1) or measured HM macronutrient content (Group 2) (Cardoso M, et al. Nutrients 2023;15:1533). Only infants with serum phosphate measured while feeding fortified HM (defined the exposure period) were included in this analysis. Low serum phosphate levels were used as an early biochemical marker of MBD and length growth as a surrogate of bone growth. Between-group differences were assessed regarding energy, protein, protein-toenergy ratio, fat, carbohydrate, calcium, phosphorus, and magnesium intakes, serum phosphate levels, prevalence of hypophosphatemia (using both cut-offs \leq 3.72 mg/dL and \leq 5.6 mg/dL), Δ length z-scores, and length gain velocity. Independent sample t-test, Mann-Whitney-U test, chi-squared test or Fisher's exact test were used as appropriate, and a significance level a=0.05 was considered. Eighty-four participants were included, 35 in Group 1 and 49 in Group 2. The exposure period covered 2nd to 5th postnatal weeks. During the exposure, infants of Group 2 received higher mean fat intake (6.1 vs. 5.3 g/kg/d, p<0.001) which was associated with lower mean weekly serum phosphate levels (5.5 vs. 6.0 mg/dL, p=0.022). Although no significant differences existed in the prevalence of hypophosphatemia, weak evidence of higher prevalence was found in Group 2, in 3th week, using the



cut-off \leq 5.6 mg/dL. The length gain velocity was faster in Group 2 during the exposure, assessed by the average of mean weekly values (1.06 vs. 0.89 cm/week, p=0.003). These findings showed that infants fed fortified HM with added modular supplements (Group 2) received significantly higher fat intake and presented significantly lower serum phosphate and significantly faster length gain velocity. We hypothesize that fortifying HM with the addition of extra macronutrients not accompanied with mineral supplementation, predispose to accumulation of insufficiently mineralized osteoid, reflected by serum phosphate levels as indicator of bone mineralization, and length growth as surrogate of bone growth. Future intervention studies, using more accurate biomarkers of bone mass content and bone mineral density, are needed to confirm this hypothesis.

Keywords: bone mineralization; human milk; human milk fortification; hypophosphatemia; length growth; metabolic bone disease; modular macronutrient supplements; preterm infants; serum phosphorus levels; target fortification

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Microbiological Analysis of Borehole Water Quality ⁺

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The increasing utilization of groundwater from boreholes by individuals within their properties has raised concerns among health professionals regarding potential health risks. The presence of microorganisms or harmful substances in this water poses a significant concern. Therefore, the primary objective of this study was to conduct a comprehensive microbiological analysis of the water sourced from boreholes in the village of Santo Ovídio, Setúbal. Specifically, the investigation aimed to identify and quantify fecal contamination indicator bacteria, including total and fecal coliforms, E. coli, faecal Enterococci, Clostridium, and Pseudomonas aeruginosa, using the membrane filtration method. The research design employed was quantitative in nature, utilizing a simple descriptive approach at level I. The study included a sample size of 20 participants who provided water samples from their respective boreholes. The analysis involved the detection and quantification of the microorganisms. The findings of the study revealed that approximately 60% of the water samples collected from the boreholes in the village of Santo Ovidio contained at least one of the targeted microorganisms. These results indicate the potential for fecal contamination, suggesting a possible risk to the water consumers in the area. The identification of fecal contamination indicators in borehole water emphasizes the need for further investigation and proper management of groundwater resources in the village of Santo Ovidio. Measures to mitigate contamination should be implemented, such as regular monitoring and appropriate treatment methods to ensure the provision of safe and clean water to the community. This study contributes to the existing knowledge by highlighting the microbiological quality of borehole water in a specific geographic area, emphasizing the importance of water quality assessment for the protection of public health. The results underscore the significance of implementing measures to safeguard the integrity and safety of borehole water sources.

Keywords: borehole water, microbiological analysis, fecal contamination, groundwater, membrane filtration method



Tunneling Technique ⁺

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The demand for the treatment of gingival recession has increased recently. The combination of the coronal advancement flap and the connective tissue graft (CTG) is considered the gold standard technique, although the tunneling technique is recognised as a more conservative and aesthetic surgical approach due to the fact that it preserves the integrity of the dental papilla and does not require vertical releasing incisions. This technique is indicated for the coverage of single and multiple Miller classes I and II recession with moderate depth. A healthy, non-smoking female patient presented with a Miller Class I gingival recession in the region of tooth # 23. Prior to orthodontic treatment, the recession was treated with the tunneling technique. This technique used microsurgical instruments to prepare the recipient site. An intrasulcular incision was made with a 69 microblade and a subperiosteal tunnel was created with tunneling instruments. A CTG was harvested from the palate using the single incision technique and placed in the recipient area using a 5/0 nylon suture. Several horizontal suspensory stitches were made using the same suture thread. Post-operative care instructions were given, and the patient was medicated with 600 mg ibuprofen (every 12 hours) and 0.2% chlorhexidine mouthwash. The suture at the donor site was removed 7 days after surgery and the suture at the recipient site was removed 15 days after surgery. In addition to the aesthetic benefits, the tunneling technique has other advantages for the patient, such as better blood supply to the graft-receiving area, faster healing, and less morbidity in the postoperative period. Removal of the connective tissue graft from the palate using the single incision technique allows the palate to heal by first intention, reducing morbidity. In this case, we were able to achieve complete coverage of the recessions and good integration with the adjacent tissues in terms of colour, volume and texture. At the same time, probing depths are < 3mm and there is no bleeding on probing. Disadvantages of this technique are the increased operating time and the fact that it is a very sensitive and delicate technique. The tunneling technique proved to be a technique with excellent esthetic results. It was observer complete root coverage an increase in the amount of keratinised gingiva.

Keywords: gingival recession; coronal advancement flap; connective tissue graft; tunneling technique.



Before surgery



3 weeks after surgery



Personality and Aggressive Behavior in a Domestic Violence Suspects Sample ⁺

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The Five-Factor Model (FFM) is the most modern and widely acknowledged method of describing and evaluate personality. According to the FFM, the five basic domains that incorporate personality are Neuroticism, Extraversion, Openness to Experience, Agreeableness, and Conscientiousness. Personality plays a significant role in predicting aggressive behavior. While developing the Aggressive Questionnaire, Buss, and Perry (1992) assumed that this behavior could be categorized into four factors: Verbal Aggression; Physical Aggression; Anger; and Hostility. Some authors (e.g., Gallo & Smith, 1998) concluded that, first, there is a correlation between neuroticism and all four measures of the Aggression Questionnaire. Second, hostility and verbal aggression were mostly unrelated to (poor) conscientiousness, although physical aggression and anger were highly related with it. Low agreeableness, low conscientiousness and high neuroticism have previously been associated with aggression (Jones at al. 2011; Jiang, 2022). Higher BPAQ scores were positively associated with neuroticism and negatively associated with agreeableness and conscientiousness (Dam et al., 2021). The goal of this study is to show the relationship between the five-factor model of personality and aggression in 54 suspects of domestic violence ($n_{men} = 46$ (85.2%); $n_{women} = 8$ (14.8%)), aged between 23 and 68 years old (M = 45.39, sd = 10.36) assessed in the Victims Information and Assistance Office (GIAV), inserted in Public Prosecutor's Office. Most of the sample were only suspects (n = 35) and others were victims and suspects simultaneously (n = 19). The relationship between victims and our sample were: 19 married; 14 ex-boyfriends/girlfriends; 11 divorced; 3 boyfriends/girlfriends; 3 partners; 3 ex-partners; 1 lover. Data were collected from lawsuits, semistructured interviews, collateral information, and clinical and forensic assessment tools as NEO-PI-R and BPAQ. The results show us a positive correlation between neuroticism and physical aggression, anger, hostility, and total aggression score; a negative correlation between extraversion and physical aggression; a negative correlation between agreeableness and physical aggression, anger and total aggression score, and a negative correlation between conscientiousness and physical aggression, anger, and total aggression score. These results are consistent with the findings of the empirical studies mentioned, namely the relation between FFM and aggression. People with higher levels of Neuroticism are more likely to experience negative emotions and engage in aggressive behaviors and people with higher levels of Agreeableness and Conscientiousness are more likely to experience positive emotions and are less likely to engage in aggressive behaviors. Our results show the importance of studying the relation between personality and aggressive behavior and allow to understand and to find assessment strategies (e.g., personality and aggressive behavior assessment) and prevention strategies for domestic violence. Therefore, we reinforce the relevance of continuing the study of this topic, which could strengthen a closer articulation between Forensic Psychology and Law and a better understanding about domestic violence offender's characteristics.

Keywords: Personality; aggressive behavior; five-factor model; domestic violence



Aggression, Genetics, and Adverse Childhood Experiences: in a university sample⁺

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The literature indicates that aggression in adulthood is associated with adverse childhood experiences and genetics. The present study aims to investigate the phenomenon of aggression in adulthood and how it is related to genetics (dopamine and serotonin polymorphisms) and adverse childhood experiences. The sample was collected as part of the research project "Aggression and Genetics in a University Context", so it is a sample of volunteer university students who participated in the general project. This sample consists of 93 individuals, of which 12 are male (12.9%) and 81 are female (87.1%), aged between 17 and 40 years (M=20.95; SD=2.98). Participants completed a protocol consisting of a sociodemographic questionnaire, Buss-Perry Aggression Questionnaire - Short Form (BPAQ-SF), Reactive/Proactive Aggression Questionnaire (RPQ), Adverse Childhood Experiences (ACE). Significant differences were found between males and females for anger, with females scoring higher than males. Genetically, there was a significant difference between 5-HTTLPR and emotional abuse. Thus, heterozygous individuals (ID) (63.6%) have more indicators for the presence of a history of emotional abuse. Statistically significant correlations were obtained between experiences are predictors of aggression in adulthood.

Keywords: Aggression, Adverse Childhood Experiences, Serotonin, Dopamine



Genetics: exploratory study for a university population ⁺

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Aggression is defined as deliberate behavior that is intended to cause pain or harm to another person. This behavior can be physical, psychological, or verbal. Aggression is not only intentional but also interpersonal. In the literature, aggression has been associated with adverse childhood experiences, personality, stress, and genetics. Aim: To contribute to an integrated analysis of the concept of aggression and its relationship with the genetic component, considering the general model of aggression. Participants: The sample consists of 93 university students aged between 17 and 40 years (M=20.9, SD=2.9). Of the 93 participants, 12 are male (12.9%) and 81 are female (87.1%). The sample was collected as part of the research project "Aggressiveness and genetics in a university context". 2) Participants completed a battery of psychological tests consisting of a sociodemographic questionnaire, the Childhood History Questionnaire (ACE) assessing history of adverse childhood experiences, the NEO-Five Factor Inventory-3 (NEO-FFI-3) assessing the five dimensions of personality, the Perceived Stress Scale-10 (PSS) assessing global stress index, the Buss-Perry Aggression Questionnaire - Short Form (BPAQ-SF) assessing forms of aggression, and the Reactive/Proactive Aggression Questionnaire (RPQ) assessing reactive and proactive aggression. Genetic material was also collected by buccal swab to investigate serotonin and dopamine polymorphisms associated with aggression. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Eqas Moniz School of Health and Science. The subscale Adverse Experiences Against the Individual showed statistically weak positive correlations with forms and functions of aggression. The Neuroticism and Agreeableness dimensions showed statistically positive and negative correlations respectively with the forms and functions of aggression. The Global Stress Index showed statistically moderate positive correlations with the forms and functions of aggression. An association was found between the variables of adverse childhood experiences, personality and stress and the forms and functions of aggression as defined by the General Model of Aggression. No effect of genetic variables was found. Limitations related to the nature of the sample studied (mostly female) may explain the results obtained.

Keywords: General Aggression Model, Adverse Childhood Experiences, Personality, Stress, Genetics, Serotonin, Dopamine



Dracaena draco L. uses, phytochemistry and medicinal activities ⁺

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The genus Dracaena belongs to the family Asparagaceae, containing about 113 species. Dracaena draco L., is the most studied species in terms of phytochemistry and pharmacological activities, with the greatest number of ethnopharmacological uses of its resin, known as "dragon's blood". Accuracy is needed cause other plant resins are also known with that name. The subspecies draco is mainly distributed in Macaronesia (Azores, Madeira, Canary Islands and Cape Verde). The Dragon Tree (D. draco L.) is an arboreal species, growing slowly, with multiple trunks and a rosette of leaves at the end of branches, forming a dense umbrella shape. The leaves are long, greenbrownish, and red at the base. The flowers form white clusters, and the fruits are orange berries with a single seed. When wounded or cut into branches or trunks, it secretes the dark red exudation called dragon's blood. This resin was very valuable in ancient times for artistic and medicinal purposes and was included in the European Pharmacopoeia. It has an astringent effect and was used as an antidiarrhoea and haemostatic agent. Overuse has led to its decline in the wild and it is now listed as vulnerable on the IUCN Red List of Threatened Species, making propagation useful. Phytochemicals present are predominantly phenolic compounds, mainly flavonols, which confer most of its described biological activities, specifically antioxidant, antimicrobial, anti-inflammatory and analgesic, cytotoxic, and antithrombotic capacities. Terpenoid compounds, namely carotenoid derivatives from the fruits, and steroidal saponins from the bark have been investigated in vivo for their cytotoxic activity on colon cancer and myeloid leukaemia cells. We performed studies for potential biotechnological production of medicinal secondary metabolites. Previously (Simões-Costa et al., 2014). we have reported successful calli induction from leaf for new biomass and secondary metabolite production. We regenerated new plants germinated in vitro, in Shultz media, with 8% agar, from seeds disinfected with 70% (v/v) ethanol and natrium hypochlorite 10% (v/v) for 20 minutes. Seeds were obtained from 400 years old tree of D. draco, growing in Jardim Botânico da Ajuda, Lisbon. Obtained plantlets were acclimated in an 8:16 h photoperiod and maintained indoor since. Aiming future safe application of its extracts and secondary metabolites, we used leaves and bark to prepare extracts, after lyophilization and grounding, with different methods: Santos et al. (2011) for organic acids, phenolic and terpene compounds, Gonzalez et al. (2003) for steroidal saponins. Total antioxidant activity was determined on the aqueous extracts of leaves and bark, through phosphomolybdate assay (respectively 83,2 and 84,4 mg ascorbic acid equivalents/g DW) and DPPH assay (28,2%. and 32,9% inhibition). In order to test their anti-viral activity, citotoxicity of the extracts was controlled through the alamarblue test, with human epithelial cells TZM-bl, being considered non-toxic (100 ug/ml), allowing further testing with this model. The characteristics of anti-oxidant activity and non-toxicity found for this species extracts are favourable for possible biotechnological and medical applications but it will be necessary to further study safety and efficacy of extracts and medicinal compounds.

Keywords: Dracaena draco L.; Dragon's blood; Phytochemistry; Medicinal activities.



Cyberaggression, personality and genetics⁺

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Cyber aggression can be defined as a form of aggression in which the perpetrator uses digital media to harm a person or group of people. The literature has linked aggression with personality and genetics. One of the models that tries to operationalize this relationship is the General Model of Aggression. The aim of this study is to analyze the relationship between cyber aggression and personality traits, in the presence of some polymorphisms associated with serotonergic and dopaminergic processes, which are related to aggression. The sample is composed of 93 individuals aged between 17 and 40 years (M=20.95; SD=2.98) in which 12 individuals are men (12.9%) and 81 are women (87.1%). The sample was collected within the scope of the research project "Aggressivity and genetics in a university context", so it is a normative sample made up of volunteer university students who participated in the general project. Participants were asked to respond to a protocol consisting of a sociodemographic questionnaire, the shortened version of the Buss-Perry Aggression Questionnaire (BPAQ-SF) that assesses forms of aggression, the Reactive-Proactive Aggression Questionnaire (RPQ) which assesses reactive and proactive aggression, the Cyber-Aggression Typology Questionnaire (CATQ) which assesses the various dimensions of cyber aggression and the NEO Five-Factor Inventory-3 (NEO-FFI-3) which assesses the 5 dimensions of personality. Together with the battery of questionnaires, the participants' genetic material was collected. Agreeableness showed significant and negative correlations with all subscales that define cyber-aggression. A significant and positive association was found between cyber-aggression and the forms and functions of aggression. Significant differences were found between men and women for aggression (Anger), with women showing higher values than men. Conclusions: Cyber-aggression and aggression are related to personality. The impact of genetic variables was not observed.

Keywords: Aggression, Cyber Aggression, Personality, Genetics, Serotonine, Dopamine



Hamstrings/ quadriceps ratio in trail runners: a preliminary study on overload and knee pain ⁺

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Studies conducted with runners with unilateral overuse injuries suggest that lower limb asymmetries are an important risk factor. It is possible to find bilateral asymmetry rates reaching magnitudes of up to 10% in isometric and isokinetic torque production of the knee. Possible reasons for such asymmetries are muscle weakness, impaired muscle coordination, and muscle strength imbalances. Biomechanical changes due to fatigue lead to suboptimal movements that may increase the risk of injury. The present study aimed to identify the isometric hamstrings/quadriceps (H/Q)strength relationship and bilateral asymmetries in Trail Running athletes. Thirty-eight amateur Trail Running athletes of both genders, ranging in age from 26 to 60 years, were evaluated. Strength was measured using the Kinvent hand dynamometer, which is a battery-operated device for measuring peak force. It is a strain gauge type dynamometer based on load cells, where a force distorts a strain gauge and converts it into a recorded electrical signal that represents the measured force in a mobile device application. The Kinvent measures force in kilogram-force (Kgf) or newtons (N) at a frequency of 75/300Hz. Assessors were trained and instructed in the use of the Kinvent hand dynamometer prior to data collection to ensure measurement competence and effectiveness. Participants were assessed at the Laboratory for Physical and Functional Assessment in Physical Therapy (LAFFFi), individually, in a room with temperature between 21°C and 23°C and humidity between 40% and 60%, in the morning hours between 9 am and 1 pm to avoid interference from the circadian cycle. During the evaluations, the participants were instructed to perform the maximal voluntary isometric contraction that was later used to assess isometric strength. The flexion (prone) and extension (seated) test with the knee at 90 degrees was performed by the examiner holding the dynamometer stationary while the participant exerts a maximum force against it. Participants were instructed to perform three consecutive maximal efforts, each lasting 5 seconds, with a 30 second rest interval between repetitions to avoid fatigue. The Shapiro-Wilk test was applied to verify the normality of the distribution of all variables in the groups of interest. The association between variables was evaluated using Pearson's correlation coefficient. Statistical inference was conducted at a 5% significance level using SPSS 28.0 software. Women have 25% and 29% smaller quadriceps and hamstrings, resulting in a reduced H/O MS ratio compared to men. Pearson's correlations demonstrate that there is no association between H/Q ratio and handgrip strength. It was possible to conclude that the identified values of the isometric H/Q force ratio were much lower than 50% bilaterally in both sexes and the bilateral asymmetry indices for the appreciable knee torque revolves around the mean value close to the threshold value of 10%.

Keywords: trail running, dynamometry, hamstrings, quadriceps, isometric

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Association between oral health values and periodontal health: an observational study ⁺

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Periodontitis is one of the most common diseases affecting the oral cavity. It is a chronic condition that involves an inflammatory response in the periodontal tissues adjacent to the tooth and can lead to a plethora of irreversible situations, including tooth mobility, tooth loss and bone loss. The aim of the observational study was to explore the relationship between periodontal status and the Portuguese version of the Oral Health Value Score in a population of adults. Using a comprehensive assessment tool and self-reported measures of periodontitis and dental caries experience, we surveyed those who sought a first appointment at the Clinica Dentaria Egas Moniz (CDEM). Participants completed a questionnaire comprising a Sociodemographic questionnaire, the Oral Health Value Scale (OHVS-PT), the Oral Health Impact Profile (OHIP-14) and a self-reported measure of periodontitis. Caries experience was assessed by clinical observation using the DMFT Index, combined with orthopantomography for more accurate results. Due to the non-normal distribution of the sample, the data were analyzed using descriptive statistics and non-parametric tests. We ended up with a final sample of 380 participants with a mean age of 43.3 years and a range of 18-90 years old. The outcomes show a positive association between Oral Health Value Scores and better periodontal health, which was evaluated with the self-report measure of periodontitis, with patients with a self-reported periodontitis score of "1" (self-reported positive) displaying lower OHVS scores in all 4 subdomains when compared with a score of "0" (self-reported negative). There were also differences when analyzing this but divided by gender, with women reporting significantly higher results when compared to men in all 4 domains of the OHVS. This study provides evidence of an association between oral health values and periodontal health, highlighting the importance of promoting oral health values and attitudes towards oral health care in both the preventative and curative management of the periodontal disease. Ultimately, this research highlights the importance of oral health values and attitudes toward oral health care in the prevention and treatment of periodontal disease. These questions are an important tool for screening and managing patients on a larger scale without needing a full triage.

Keywords: Periodontal Disease; Periodontitis; Oral Health Value Scale; Periodontal Health.



Wernicke-Korsakoff syndrome after bariatric surgery: Case study ⁺

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Vitamin and mineral deficiencies are prevalent nutritional disorders following bariatric surgery. Although they are more prevalent after malabsorptive procedures such as bypass, they also occur in restrictive procedures such as gastric sleeve. The mechanisms leading to the occurrence of these deficits are related to the presence of poor nutritional intake or poor adherence to multivitamin and multimineral supplementation. Wernicke-Korsakoff syndrome (WKS) is an acute neurological disorder resulting from thiamine (vitamin B1) deficiency. This syndrome is composed of two distinct phases: first, Wernicke Encephalopathy (WE), the acute phase of this syndrome, that is characterized by a triad of altered mental status, ocular signs, and ataxia, followed by the chronic phase of WKS, called Korsakoff's Syndrome (KS) that is known by the presence of anterograde amnesia and confabulation. We aim to report a case of a patient with Wernicke-Korsakoff syndrome after bariatric surgery. Patient's retrospective chart review was done in order to retrieve the relevant clinical data. The patient was a 24-years-old, female, with a BMI of $48kq/m^2$ that was submitted to a sleeve gastrectomy surgery for morbid obesity. During the following 2 months, the recovery from surgery was complicated by non-specific symptoms such as nausea, recurring vomiting and a significant decrease in food intake that led the patient to go to the emergency room six times with hospitalization on the last occasion for a definitive diagnosis. During the fifteen days of hospitalization, the patient developed ocular diplopia, nystagmus, complaints of rotatory vertigo and gait abnormalities. A magnetic resonance imaging of the head was performed which revealed no significant changes. After formal neurological assessment, treatment with parenteral thiamine (100 mg, 3 times a day) was started, without prior dosing. The clinical improvement observed confirmed the diagnosis of Wernicke-Korsakoff syndrome. Bariatric surgery is intended to help obese patients attain a healthier body weight. Bariatric surgery may contribute to vitamin deficiency and therefore to Wernicke-Korsakoff syndrome. The variability of clinical presentations and the low specificity of neurological signs can lead to under or late diagnosis of this pathology, resulting in a lower likelihood of full recovery. Education on the adverse consequences of malnourishment is mandatory before and after the surgery. Investigation of nutritional deficiencies in both pre and postoperative period is crucial in order to prevent complications such as Wernicke-Korsakoff syndrome.

Keywords: Wernicke–Korsakoff Syndrome; Thiamine; Bariatric surgery; Thiamine deficiency; Nutritional status; Sleeve gastrectomy; Obesity.



Developing an in-house GC-MS method to measure THC and THC-COOH in cannabis samples⁺

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Being the most widely consumed illicit drug worldwide, it is vital that cannabis samples go through certain monitoring procedures that will provide knowledge regarding the compounds present in the plant, as well as their respective content. The lack of a standard method to analyze herbal cannabis samples, led to the present study, with the aim of creating an in-house GC-MS method to understand the Δ^9 -THC and Δ^9 -THC acid content in cannabis. The method starts with a solid-liquid extraction procedure on herbal cannabis plant using methanol. Identification and quantification were achieved by GC-MS calibration curves with standard solutions for Δ^9 -THC and Δ^9 -THC-COOH. The method was tested in real samples and the obtained chromatographic peaks and mass spectra confirmed the presence of Δ^9 -THC and Δ^9 -THC-COOH in the seized sample. The sample's content of Δ^9 -THC was 5,21%, whilst the content of Δ^9 -THC-COOH was non-significant (0,35%). The seized sample had a low content of Δ^9 -THC in comparison with the average European Δ^9 -THC content of 11% in herbal forms of cannabis estimated by the 2022 European Report on Drugs. In conclusion, the method allowed to obtain reliable results regarding qualitative and, especially, quantitative analysis, given that the value obtained for the determination coefficient (R²) of the calibration curve corresponded to 1. Further analysis must be carried out in order to validate the method.

Keywords: Cannabis, Gas Chromatography; Mass spectrometry; THC content



Alexithymia, Personality, Internet Addiction, and Interpersonal Relationships ⁺

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Alexithymia is a clinical concept used to describe individuals with significant difficulty or even an inability to express their emotions and meanings. Studies suggest that alexithymia is a multifactorial personality trait associated with deficits in cognitive-emotional processing, often observed in various disorders involving difficulties in identifying, verbalizing, and experiencing emotional situations. Personality plays an important role in the lives of subjects who, consequently, have different personalities, in this way, they experience their emotions differently. A positive association was found between alexithymia and neuroticism, the latter being considered the greatest predictor of alexithymia. A negative association was found between alexithymia and extraversion, openness to experience and conscientiousness. The dimension agreeableness is associated with lower levels of alexithymia. Individuals with alexithymia may use the internet as a tool for social interaction to better regulate their emotions and fulfill their social needs. Thus, individuals with alexithymia are more likely to be addicted to the internet than individuals without alexithymia, as they use it as a tool for emotional expressing. It becomes essential to study the relationship between the two variables, and how internet use is perceived by individuals with alexithymia. The causal relationship is not yet clear due to the interaction of numerous other variables that may influence this relationship. The main goal is to demonstrate how alexithymia and internet dependence consequently affect interpersonal relationships in university students. The sample consists of young university students. The sample consists of 491 individuals, of whom 119 are male (24,2%), and 372 are female (85.8%). Participants completed a battery of psychological tests: a) a sociodemographic questionnaire; the Toronto Alexithymia Scale (TAS-20); the Internet Addiction Test (IAT); the Neo-Five Factor Inventory (NEOFFI) and the Interpersonal Behavior Scale - Reduced Version (ECIr). The psychological assessment battery was applied in a presential way. Based on the results of the presented research, alexithymia and internet dependency can negatively impact interpersonal relationships as they disrupt emotional expression, communication, and social engagement. Also, the relationship with the personality indicates that more neurotic, extrovert, less agreeable and less conscientious individuals reveal a higher degree of internet addiction and Alexithymia. The results obtained confirm the data already presented in the literature. Gathering more data and gender differences analysis are the next research step. The development of prevention programs and social skills training strategies for young adults considering individual differences and the challenges of the internet use are the most import contribution of this project.

Keywords: alexithymia; internet dependency; personality; interpersonal relationships

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Symptomatology in Intimate Violence Offenders and Victims[†]

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Domestic violence is a major social problem with severe consequences for everyone. It consists of every type of violence such as physical, sexual, emotional, psychological, economic, and negligence, perpetrated in family. According to the World Health Organization (WHO), 30% of women around the world have been victims of domestic violence in their life, and 27% of women between 15 and 49 years old have been battered by their intimate partner. Intimate partner violence (IPV) is a form of domestic violence that consists of any action that holds the intention to inflict physical, sexual and/or psychological damage to the offender's partner. So IPV continues to represent most crimes committed in Portugal and constitutes a large problem that can have detrimental effects on families as well as on the larger community. Although there is no direct relationship between psychopathology and IPV, it is common to identify personality and psychiatric disorders in several individuals judged by the criminal justice system. Based on studies on IPV offenders and victims' symptomatology, this study aims to identify psychopathological symptoms in IPV offenders and victims. Our sample consisted of 59 perpetrators and 63 victims of IPV, whose evaluation was requested by court order. The information on the evaluation was obtained through the database of the Forensic Psychology Office [Egas Moniz Forensic and Psychological Sciences Laboratory (LCFPEM)]. We used the Brief Symptom Inventory (BSI), a self-report measure in which respondents' rate, in the past week, several symptoms used to identify self-reported clinically relevant psychological symptoms. This study demonstrates that victims and offenders have active symptoms, with significant differences in five psychopathological dimensions, namely somatization, obsessivecompulsive, anxiety, phobic anxiety and psychoticism, and victims present a complex exhibition of symptoms. The impact of victimization is revealed in negative repercussions on the level of global functioning in IPV victims rather than IPV offenders. Among the dimensions examined, victims consistently score higher than offenders. Although no similar effect can be inferred, the relevant literature has consistently identified an objective relationship between vulnerability resulting from exposure to violent behavior in victims and offenders and the emergence of psychopathological conditions. Furthermore, the symptoms observed align with those commonly described in the literature, where major depression, anxiety and dependency/addiction are deeply related to the context and dynamics of abusive situations. In addition to aiding assessment, these findings contribute significantly to more effective interventions. They assist professionals in adapting existing programs to obtain better results in preventing relapses. Consequently, interventions can specifically focus on factors that increase the risk of committing this type of violence. Despite the results obtained, we are aware that this investigation has some limitations, namely the samples size, which may influence the results, which prevent us from drawing more valid data analysis and conclusions. Another limitation is the evaluation of psychopathological by self-reported measures, which has been criticized since these do not cover all information processing strategies.

Keywords: Intimate partner violence; symptomatology, offenders, victims



The influence of polymorphisms MMP8 -799 C>T and MMP9 -1562 C>T towards periodontitis susceptibility in a Portuguese population of Egas Moniz Dental Clinic users [†]

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During the inflammatory process of periodontal disease, the periodontium is destroyed, resulting in increased levels of various types of proteolytic enzymes in the tissue, including matrix metalloproteinases (MMPs). These are a family of structurally related but genetically distinct enzymes capable of degrading almost all extracellular matrix (ECM) proteins. Several studies have been conducted to understand the association between the MMPs' genetic components of the host and the risk of developing this disease, namely the MMP8-799 C>T and MMP9 -1562C>T polymorphisms. Both genes contain an SNP in the promoter region at position -799 and -1562, respectively, where a cytosine to thymidine substitution occurs. Functionally, the T allele of these variants can eliminate a binding site for a transcriptional repressor, thereby increasing both MMPs 8 and 9 expression and consequently, their transcriptional activity. These polymorphisms have been the subject of numerous studies to explore and focus on their association with periodontal disease, but inconsistent results have been found. Our aim was to characterize the relationship between the presence of polymorphisms of metalloproteinase 8 (-799 C>T) and 9 (1562 C>T) and the development of periodontitis through a pilot study carried out in a Portuguese population of users of the Egas Moniz Dental Clinic. The study was approved by the Egas Moniz Ethics Committee. A sample of 45 individuals was used, of which 21 were periodontal patients, and the remaining 24 were periodontally healthy. The samples from the jugal mucosa were collected with an OmniSwab®, for later DNA extraction. Genetic analysis for the MMP-8-799 C>T and MMP-9-1562 C>T polymorphisms was performed by PCR (Polymerase Chain Reaction) and RFLP (Restriction Fragment Length Polymorphism) using SfcI and SphI respectively, followed by electrophoretic analysis. Statistical analysis was performed using the R Statistical 4.0 software (R package based on the χ^2 test, Statistical Computing and Graphics, Inc.). Our results showed that all our polymorphisms were in Hardy-Weinberg equilibrium. The allelic and genotypic distributions for MMP9 -1562 C>T were the same in periodontal patients and controls (p = 0.905 and 0.911, respectively), whereas there were significant differences for MMP8 -799 C>T, (p = 0.040 and p = 0.024, respectively). We conclude that there is no association between the simultaneous presence of the mutation in both genes and the presence of periodontal disease and that there is an association between the MMP8 -799 C>T mutation and periodontitis.

Keywords: MMP Polymorphisms; Periodontitis; Molecular Genetics

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P2 Living in a health promoting environment

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Parkinson's related Pain symptoms in recently diagnosed individuals with Parkinson's disease ⁺

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Pain is a common nonmotor symptom of Parkinson's, with a prevalence of 67.6 % for PD-related pains that increases the longer people have Parkinson's. It has a significant negative impact on the quality of life. However, distinguishing PD-related pain from non-PD-associated pain can be challenging, but it is critical to get to the right treatment strategy timely and accurately. An association with the disease (course of PD, impact of fluctuations, and medication) should be determined before evaluating therapeutic options. Objective: To determine the frequency, characteristics, and impact of Parkinson's symptoms related to pain in recently diagnosed people using a questionnaire. Pain assessment in Parkinson's is predominantly reliant on subjective data encompassing factors such as intensity, location, duration, relationship to medication use, and potential underlying causes. This understanding facilitates more informed decision-making in guiding intervention selection, ultimately expediting the path to pain resolution and help professionals identify education needs. With practical education programs, people with PD can learn to closely monitor their pain symptoms (e.g., pain diaries), allowing them to better report it to the neurologist, as well as identify patterns or triggers that can help inform better management strategies. A qualitative online survey was developed by rehabilitation specialists and e-mailed to all participants. Twenty-six people with PD (22 diagnosed with less than 5 years) with a mean age of 72 ± 8.1 years, completed the survey. Our results showed that over the past month, 11.5% had no uncomfortable feelings (normal), and 88.5% had uncomfortable feelings in their body, like pain, aches tingling, or cramps. Of these, 47.8% had these feelings but could do things and be with people without difficulty (slight), 34.8% had these feelings causing some problems when they did some daily activities or were with other people (mild); 8.7% had these feelings causing many problems, but they do not stop them from doing things (moderate); and 8.7 % had these feelings stopping them from doing things (severe). The 5 main types of pain-related symptoms most frequently reported included: 53,8 % pain around the joints (including pain related to arthritis), 19.3 % pain related to jerking leg movements during the night, which improves with movement (restless legs syndrome), 15.4% pain related to a specific internal organ (pain around the liver, stomach or bowels); 11.5% shooting pain/pins and needles down the limbs; and 11.5% painful muscle cramps in a specific region during "off" period. Almost 91% referred not knowing if the pain was related to their Parkinson's diagnosis. Only 52.2% said they discussed their pain symptoms with their neurologist/movement disorder specialist. Most participants reported some pain-related symptoms and expressed concerns about not knowing if it was related to Parkinson's. Educational programs play an essential role in informing patients and care partners on how to distinguish their PD-related pain from normal aging types of pain, how to address it, and how to keep an accurate record. This is vital in helping doctors provide the best possible care for their health needs.

Keywords: Parkinson's disease, Pain, Recently diagnosed, Non-motor symptoms



Development of an immune-inflammation index for prediction of periodontitis based on blood circulating markers[†]

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It is estimated that between 2011 and 2020, approximately 62% of dentate adults were affected by periodontitis, with severe periodontitis accounting for 23.6% of cases. Clinically, cases of periodontitis often show a marked local inflammatory cell infiltrate and a pro-inflammatory plethora that translates systemically through the bloodstream. Several prognostic attempts have been made in periodontitis, but an indicator based on circulating markers, which may better reflect the balance of the inflammatory and immunological state of the host, has not yet been reported in periodontitis. A new systemic immuno-inflammation index (SII) has been proposed to predict the prognosis of several diseases. With this index in mind we have reason to believe that this index could be explored in the detection of clinically diagnosed cases of periodontitis. Therefore, the aim of this study was to develop and evaluate an immuno-inflammation index based on circulating markers in the blood for the prediction of periodontitis. Participants with complete and valid oral health status (periodontal) from the National Health and Nutrition Examination Survey (NHANES) 2009- 2010, 2011-2012 and 2013-2014 were enrolled. Full-mouth periodontal data was carried out through a calibrated process for gingival recession and periodontal pocket depth (PPD) as previously described. According to the protocol of NHANES 2011-2014 cycle, the Beckman Coulter method of counting and sizing, in combination with an automatic diluting and mixing device for sample processing, and a single beam photometer for hemoglobinometry was used for counting blood cells from peripheral blood samples. Using data from a final sample of 9709 participants who fulfilled eligibility criteria, with a mean age of 49 years and some with systemic diseases such as diabetes, hypertension and chronic medical conditions, the SII was applied to the study sample, finding that patients with and without periodontitis showed little different scores (518.69 vs 519.86, p=0.912). Analyzing the PCA distribution of the variables, according to the rotated component matrix, we found some patterns forming clusters among the variables. In the multivariate analysis, 6 variables had significant predictive value towards the presence of periodontitis (p<0.001): basophils, red blood cells, hemoglobin, mean corpuscular volume, mean platelet volume and red blood cell distribution width. We were able to propose a new index of immuno-inflammation for periodontitis that when compared with SII showed a high significance. The proposal of this index is designed according to the new periodontal classification to predict the degree and stage of periodontal disease, which recognizes the importance of risk factors and comorbidities associated with periodontal disease, as well as the importance of early diagnosis and personalized treatment.

Keywords: periodontitis; circulating markers; systemic immuno-inflammation index.


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Invasive fungal infections are increasing, and Candida spp. are the main cause. Yeasts species other than Candida albicans are becoming more frequent, and some of them may have variable patterns of susceptibility to antifungal agents, making it important to identify them correctly. In most cases, yeasts identification to the species level allows to predict their drug susceptibility but conventional identification methods may present some drawbacks. Molecular amplification methods [Polymerase Chain Reaction (PCR)] and proteomic analysis by Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) techniques have emerged as an alternative's methods. The aim of this study was to evaluate the concordance of the identification of clinical isolates from Intensive Care Units (ICUs), at species level, by culture methods based on standard morphological and biochemical criteria, MALDI-TOF MS and PCR. During a two-year multicenter prospective observational study in ICU patients from two tertiary hospitals located at Lisbon metropolitan area, 988 axillar/inquinal swabs were taken to unveil the prevalence of C, auris at Portuguese ICUs. This investigation has been approved by the Institutional Ethical Board of all institutions enrolled. All swabs' isolates (n=371) were plated on Sabouraud Dextrose Agar and chromogenic agar (CHROMagar®). Filamentation test, enzyme profile and sugar assimilation pattern were used for presumptive identification. PCR assays was optimized for C. auris and Candida cryptic species identification. MALDI-TOF MS methods analysis with Vitek-MS® (bioMerieux, France) system was used for a definitive identification. The following species presumptively identified by phenotypic methods were studied: C. albicans complex (n=183), C. parapsilosis complex (n=115), C. glabrata complex (n=39), C. tropicalis (n=15), C. guilliermondii (n=3), C. famata (n=2), C, kefyr (n=1), Saccharomyces cerevisae (n=1), Rhodotorula sp. (n=9) and Trichosporon sp. (n=3). ATCC collection strains C. parapsilosis 22019, C. glabrata 15126, C. albicans 90028 and C. auris DSMZ 21087 were included in the study. Correctly identification of the 371 isolates by MALDI-TOF MS included: C. albicans (n=185), C. parapsilosis complex (n=112) [C. parapsilosis sensu stricto (n=109), C. orthopsilosis (n=2), C. metapsilosis (n=1)], C. glabrata (n=36), C. tropicalis (n=15), C. lusitaniae (n=4), C. guilliermondii (n=3), S. cerevisae (n=1), R. rubra (n=9), T. inkin (n=5) and T. asahii (n=1). The direct concordance between the conventional identification method and MALDI-TOF MS was 92% (341/371). Discrepancies were observed with the following species: C. parapsilosis; C. glabrata; C. tropicalis; C. guilliermondii; C. famata and C. kefyr. There was a 100% correlation between MALDI-TOF MS and PCR assays for identification of cryptic species of C. albicans, C. parapsilosis, C. glabrata complexes and C. auris. These results allow us to conclude that conventional methodologies are still useful to reliably identify the most frequently isolated species from clinical samples, but when dealing with rare, uncommon, or cryptic Candida species, it is important to confirm them using technologies such as MALDI-TOF MS. The PCR assays used allowed a reliable species identification and has the potential to be implemented into epidemiological studies to broaden the limited knowledge of cryptic species complexes.

Keywords: Candida spp.; MALDI-TOF MS; PCR; Yeasts; Identification

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Periodontal disease and musculoskeletal injuries in athletes – An exploratory study [†]

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Our aim was to analyze the possible relationship between musculoskeletal health and periodontal health in athletes. Forty-one athletes who attended the Sports Dentistry Consultation of the Egas Moniz Dental Clinic, from February 2022 to June 2022, were invited to participate in this observational study. After recording and analysing the clinical parameters: oral hygiene index, bleeding on probing, probing depth, clinical attachment loss and community periodontal index, participants were divided into three groups (healthy, with gingivitis and with periodontitis). Prior to the clinical evaluation, saliva samples were collected and frozen at -80°C until the concentrations of IL-1β and IL-6 were determined by Enzyme-Linked Immunosorbent Assay (ELISA) method. To assess the athletes' musculoskeletal health, Nordic Musculoskeletal Ouestionnaire, a musculoskeletal injury self-perception questionnaire was applied. Of the total number of athletes (13 female and 28 male, mean age of 24.6 ± 5.9 years), 70.7% had gingivitis, 14.6% had periodontitis and 14.6% were healthy. Higher number of musculoskeletal injuries were reported by athletes with periodontitis although the differences between groups were not statistically significant. Similarly, IL-1 β and IL-6 concentrations were higher, in the periodontitis subjects, but without statistical significance. No significant correlations were found between the levels of IL 1β, and IL-6 and the periodontal clinical parameters evaluated. In this study, 85.3% of athletes revealed periodontal diseases, the severity of which seems to be linked to a greater number of musculoskeletal injuries. Oral health screening and oral health promotion programs should be implemented in this population. Further studies are needed to evaluate the possible relationship in the pathogenesis of periodontitis and the development of musculoskeletal injuries.

Keywords: Periodontal disease, Musculoskeletal injuries, Athletes, Salivary markers



A Protocol for Assessment of Societal and Clinical needs of Patients with Amyotrophic Lateral Sclerosis and relationship with nutritional status ⁺

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Amyotrophic lateral sclerosis (ALS), commonly known as Lou Gehrig's disease, is a progressive neurodegenerative disorder characterized by the degeneration of motor neurons in the brain and spinal cord. This leads to muscle weakness, twitching, and eventual paralysis, affecting voluntary movements such as walking, speaking, and breathing. Individuals with ALS face various challenges, including nutritional complications and societal needs that impact their overall well-being and quality of life. This study aims to evaluate the societal needs and nutritional status of ALS patients, with ALS, over a maximum period of 18 months. The acceptance of participation in the study by the patient is necessary. Data collection will be conducted through a structured questionnaire, adapted from the model developed by researchers at the Epidemiology Unit of the Faculty of Pharmacy, University of Lisbon. The study will include patients from two clinical settings: the Nutrition Outpatient Clinic at the Portuguese Association of Amyotrophic Lateral Sclerosis (APELA), representing patients in the early stages of the disease with better nutritional status and autonomy, and the Artificial Nutrition Outpatient Clinic at Hospital Garcia de Orta (HGO), representing patients in advanced stages with poor nutritional status. The questionnaire will assess the societal needs specific to the ALS population in the Lisbon metropolitan area. Additionally, various tools will be used to evaluate nutritional status, including the Mini Nutritional Assessment® (MNA®) for nutritional risk assessment and the Global Leadership Initiative on Malnutrition (GLIM) criteria for diagnosing malnutrition. Anthropometric measurements and handgrip dynamometry will also be conducted. Meeting the societal needs of ALS patients, such as specialized medical care, financial support, emotional and psychological assistance, accessible infrastructure, public awareness, and research advocacy, can significantly enhance their quality of life. Adequate nutritional support, including dietary modifications, nutritional supplements, and enteral nutrition, plays a crucial role in managing ALS-related nutritional challenges and improving overall health outcomes.By analyzing the relationship between societal needs and nutritional status, this study aims to shed light on the interplay between these factors and their impact on ALS patients. The findings will contribute to a better understanding of the complex needs of ALS patients and the importance of addressing them holistically. Ultimately, this study aims to improve the quality of life and survival of ALS patients by identifying strategies to meet their societal needs and optimize their nutritional support.

Keywords: Amyotrophic Lateral Sclerosis; Nutritional Status; Societal Needs

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Susceptibility patterns of Candida spp. collected from Intensive Care Units: a prospective study in 2020 – 2022 [†]

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The frequency of Candida spp. isolates as a cause of hospital infections has risen in recent years, leading to high morbidity and mortality rates. In addition, the emergence of antifungal drug resistant non albicans species (NAC) such as C. auris, is causing great concern for health care systems across the globe. Most often empirical antifungals are prescribed for candidemia, mainly based on locally and country-wide antifungal surveillance data, which differs for every geographic region. Therefore, this study aimed to evaluate the antifungal susceptibility pattern of Candida spp. isolates from Intensive Care Units (ICU) patients. In this multicenter prospective observational study, 674 patients in ICU were recruited from January 2020 through December 2022. Collection of axillar/inguinal swabs was made at admission and during the ICU stay (5th and 8th day). Patient data was obtained through a form containing epidemiological and clinical information. This investigation has been approved by the Institutional Ethical Board of all institutions enrolled. Isolates were identified by cultural, Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) and molecular methods. In vitro antifungals susceptibility tests (AST) were performed for fluconazole, voriconazole, amphotericin B and anidulafungin, according to concentration gradient Etest® strip technique following the manufacturer's instructions. C. parapsilosis ATCC 22019 and C. krusei ATCC 6258 standard strains were used as quality controls. Results were interpreted based on the clinical breakpoints recommended by the European Committee on Antimicrobial Susceptibility Testing. A total of 988 samples were received from ICUs during the study period, resulting in 355 Candida spp. isolates, mainly C. albicans (n = 185). Among NAC, C. parapsilosis was the most frequent (n = 114), followed by C. glabrata (n = 36) and C. tropicalis (n = 15). Most Candida species exhibited susceptibility to antifungals. Amphotericin B, voriconazole and anidulafungin were the drugs for which all Candida species showed more susceptibility, respectively 100%, 99.7% and 97.5%. The overall rate of resistance to fluconazole was 2.3%. For fluconazole, NAC isolates were more resistant (1.4%) than C. albicans (0.8%). The current study demonstrates the prevalence and antifungal susceptibility pattern of Candida species in Portugal ICUs. Within this cohort, antifungal resistance remains uncommon among Candida spp. isolates. The rapid identification of Candida spp. isolates and standard AST are essential procedures for controlling the rise of resistant NAC spp. in the ICU setting. This investigation opens the possibility for a better understanding and management of antifungal empirical therapy within institutions, guiding therapeutic decisions.

Keywords: Candida spp., ICUs, Fluconazole, Voriconazole, Amphotericin B, Anidulafungin, resistance.

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Periodontitis induction in animal models: a systematic review and meta-analysis ⁺

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Periodontitis is a chronic inflammatory disease of the supporting tissues of the teeth, initiated by dysbiosis of the oral microbiota in a susceptible host. This public health problem affects more than 50% of the adult population worldwide. Periodontitis can lead to tooth loss, decreased quality of life and is strongly associated with a systemic immune-inflammatory response. However, the biological mechanisms underlying the pathogenicity of periodontal disease remain to be elucidated. The use of animal models allows the explore the disease onset, progression, systemic events and its treatment. A variety of periodontitis models have been established over the past few decades, although there is much uncertainty about which models are suitable for the induction of periodontitis. We aimed to summarise and compare the alveolar bone loss (ABL) of different protocols of periodontitis induction in animal models. PubMed, Embase and Web of Science were searched up to March 2023. Systemically healthy animal models of experimentally induced periodontitis with a control group reporting ABL were eligible for inclusion in this systematic review. Studies were subgrouped according to the disease induction method. Risk of bias (RoB) were appraised using the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE). Pooled estimates were made using a through ratio of means (RoM) random-effects meta-analysis. After screening 10835 articles, 261 were included. All studies performed one or more different techniques to induce periodontitis and compared them with a control group. Overall, the ligature-induced periodontitis model and the combination of ligature and periodontal inoculation with bacterial lipopolysaccharides (LPS) were the methods with the highest ABL (n=241; RoM= 2.32; 95% CI=2.16;2.49; I²=99.3% and n=8; RoM=2.32; 95% CI=1.97;2.83; I2=93.3%), followed by the LPS induction (n=36; RoM=2.06; 95% CI=1.70:2.50; $I^2=99.6\%$), the ligature-induction with oral inoculation of periodontitis-associated bacteria (n=30; RoM=2.04; 95% CI=1.73;2.43; I2=99.4%), the bacterial inoculation (n=38; RoM=1.71; 95%CI=1.38;2.11; I2=98.8%) and oral gavage (n=22; RoM=1.27; 95%CI=1.17;1.38; I2=97.6%). When analysing the 10 items of RoB, more than 80% of the included articles showed a low RoB in four items: baseline characteristics, random housing, incomplete outcome reporting and other bias. The opposite can be observed in the items: blind outcome assessors (61.5%), selective outcome reporting (100%), where a high RoB was indicated. Allocation concealment, the blinding and the random outcome assessment were not reported in any article. Overall, ligature-induced periodontitis models and the combination of ligature with LPS were the models that best performed in mimicking periodontitis and with higher ABL.

Keywords: Periodontitis induction; Animal model; Alveolar bone loss.



Stem cell modeling of cerebellar dysfunction in Angelman syndrome ⁺

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Brain organoids are becoming a popular tool to model human diseases with a neurodevelopmental cause. Specifically, guided and regionalized brain organoids can be used to study neurodevelopmental disorders such as Angelman syndrome (AS), which has no cure and is caused by the absence of functional UBE3A protein in neurons. AS is characterized by developmental delay, speech impairment, ataxia, and epilepsy. Cerebellar dysfunction has been observed in Angelman individuals and may be responsible for many of the neurodevelopmental defects associated with this disorder. However, no direct link between cerebellar dysfunction and AS symptoms has yet been established. Recently, our group optimized a cerebellar differentiation protocol that recapitulates cerebellar development and generates mature cerebellar neuron subtypes such as Purkinje and granule cells. We applied this protocol to model AS using two AS iPSC lines with the most common genetic defect in Angelman patients, a class II deletion, AG1.0, and AS-D. Two control iPSC lines were also used. The results showed that AS class II deletion-derived cerebellar organoids were consistently smaller than the controls and showed a deficit in cerebellar commitment and lower expression of progenitor markers. Transcriptomic analysis revealed downregulation of several gene sets, some of which were involved in cerebellar differentiation, and upregulation of apoptosis-related genes. The transcription signature of AS neurons showed an underrepresentation of mature neuronal markers and an overrepresentation of progenitor/glial markers, hinting at a possible delay in maturation. Functional maturation of AS class II deletion iPSC-derived cerebellar neurons was also impaired. We also investigated cerebellar differentiation in a less severe AS condition, mutation of maternal UBE3A allele, using the ASD3X cell line, and found a milder functional impairment of cerebellar neurons. To rule out genetic variability as a possible cause of this phenotype, we corrected the mutation using CRISPR/Cas9 and obtained several isogenic control cell lines. These cell lines are currently being characterized and will soon be used in parallel with the ASD3X cell line in cerebellar differentiation, providing more robust clues for the observed phenotype. The results obtained so far suggest an overall impairment of the cerebellar system in AS, with the stronger phenotype corresponding to the more severe symptomatology of the AS caused by maternal deletion. To model all genetic causes of AS, funding was obtained from Angelman Syndrome Alliance (ASA) for generation of iPSC lines from all genetic causes of AS, with genetically matched familial controls. The project aims to create a novel and robust research tool to enhance our capabilities in studying AS pathology towards therapeutic advancement. The project is being carried out in collaboration with the Portuguese Association for Angelman syndrome (Angel.pt) to recruit families for the study. In conclusion, the study sheds light on the importance of modeling all genetic causes of AS to advance therapeutic strategies, and the protocol optimization provides a novel research tool to study AS pathology.

Keywords: induced Pluripotent Stem cells; cerebellar organoids; Angelman Syndrome; disease modeling; neurodevelopmental disorder; CRISPR/Cas9; transcriptomics



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Abstract

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Evidence of acute restoration of gait variability patterns ⁺

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Synchronizing steps to external cues is a common practice in gait rehabilitation to promote motor control. However, cued walking typically presents no variability between cues, disregarding the natural fluctuations that characterize the gait patterns of healthy young individuals. The outputs of healthy locomotor systems exhibit a fractal structure in their patterns, i.e., complexity. It is, nowadays, acknowledged that complex gait patterns play an important role in human locomotion, namely, in obstacle avoidance and fall prevention. Importantly, aging is a major risk factor for a pronounced breakdown in gait complexity. Recent research has proposed the incorporation of fractal properties within the cues' pattern of display in gait rehabilitation settings, defending it shows great potential for the restoration of gait complexity in older adults. The aim of this study is to investigate the short-term carryover effects of walking synchronized to different temporally structured visual metronomes, isochronous (ISO) and fractal (FRC), on gait complexity of older adults. Twenty-two older adults were randomly distributed in two groups and underwent three treadmill trials: one uncued (OFFpre); one synchronizing to one of the two differently structured metronomes, ISO or FRC (ON); and another uncued, after a washout period (OFFpos). Gait patterns were assessed during all trials. We hypothesized that older adults would exhibit positive carryover effects after walking synchronized to the FRC metronome, but not after the ISO one. Detrended Fluctuation Analysis (DFA) was used to determine the fractal-scaling exponent (a) for the inter-stride interval time series (a-ISIs). Repeated measures analysis of variance (ANOVA) was conducted to investigate differences between groups and across conditions. a-ISIs from the group that walked synchronized with the fractal metronome was significantly higher during the ON condition and after the washout period. Walking to a fractal-like visual stimulus has a positive carryover effect on gait complexity of older adults.

Keywords: ageing; visual cueing; gait rehabilitation; gait variability; fractal patterns restoration

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Optimisation of subgingival biofilm sampling and DNA extraction protocol for shotgun metagenomic sequencing ⁺

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A clear understanding of the pathogenesis of peri-implantitis (PI) is lacking. PI is an immunemediated biofilm pathological condition and is considered the leading peri-implant disease. It is characterized by inflammation of the peri-implant connective tissue, with clinical signs of inflammation, bleeding and/or suppuration on probing, increased probing depth and progressive loss of supporting bone that can lead to implant loss. More in-depth studies, such as those using shotgun metagenomics sequencing, are essential to unveil the microbial and functional dysbiotic state in PI. Sampling subgingival biofilms from dental implants may constitute a challenge to obtain enough sample that will allow retrieving DNA with the quality and concentration required for shotgun metagenomics. Therefore, the aim of this study was to optimise the protocol for collecting subgingival samples from dental implants (both healthy or diagnosed with PI), for further DNA extraction and subsequent shotgun metagenomics sequencing analysis. Preliminary sample collections were carried out from single implants using three different methods, namely 1) four size 45 paper points, 2) a curette plus a paper point, or 3) three periopaper strips (Oraflow). All samples were placed in a Tris-EDTA buffer solution, stored at -80°C, and then processed for DNA extraction within a week. DNA extraction was performed from all samples using the DNeasy PowerSoil Pro Kit (Qiagen) according to the manufacturer's instructions, but a prior step was added to deplete human DNA, and it involved osmotic lysis in water followed by the addition of propidium monoazide to allow selective degradation of host cell DNA. Overall, the paper-point method produced more variable results and was deemed less effective (concentrations of DNA lower than 10 $ng/\mu L$). Using a curette required prior anaesthesia with local anaesthetics, such as lidocaine or articaine, and did not significantly increase the concentration of extracted DNA. Therefore, the method considered more suitable for collecting a larger amount of sample and resulting in sufficient DNA was the one using three periopapers/implant (concentrations of DNA higher than 20 ng/µL). As expected, regardless of the method, healthy implants were more challenging than PI-affected implants, because they generally harbour a less abundant subgingival biofilm. This preliminary study was essential to optimise the subgingival sampling of dental implants, as the various sampling protocols reported in the literature were revealed to be not equally suitable for our purpose, which is to extract sufficient total microbial DNA from the samples for further shotgun metagenomic sequencing analysis.

Keywords: dental implants; DNA extraction; peri-implantitis; sampling subgingival biofilms; shotgun metagenomic sequencing.

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Effect of periodontitis induction on female reproductive organs and systemic inflammatory burden ⁺

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Periodontitis is a chronic inflammatory disease with a high prevalence worldwide and is a public health problem. It is characterized by the destruction of the periodontal complex, and it is mediated by the dysbiosis between the oral microbiota and the host immune system, which can affect several organs, including the reproductive system. Nevertheless, the biological plausibility of this association remains to be investigated. The aim of this study is to understand the effects and consequences of periodontitis induction in rats on blood levels and inflammation in the female reproductive organs. Fourteen female Wistar rats were divided into a control group (n=4), and periodontitis induced by 5-0 silk ligatures on the upper 2^{nd} molars group (n=10). Within each group, half were sacrificed after 21 days and the other half on the 28th. Alveolar bone loss was evaluated by histomorphology. The inflammatory process in the periodontium and female reproductive organs was evaluated by histological analysis. Serum levels of blood counts, sex hormones and inflammatory parameters were measured and are displayed as mean ± standard deviation. Alveolar bone loss was observed in both experimental groups, particularly during the 21-day protocol. The most affected sides were the distalbuccal (at 21 days 0.67±0.17 vs 0.24±0.07) and disto-lingual (at 21 days 0.65±0.09 vs 0.22±0.05), and the less affected one was buccal $(0.47\pm0.21 \text{ vs } 0.25\pm0.02)$. In the 28-day protocol, the most affected area was disto-buccal (0.58±0.34 vs 0.26±0.02). Histological analysis showed an increase of inflammatory cells in the periodontium, mainly neutrophils, with a high inflammation score, especially at 21 days. The uterus was the female reproductive organ more affected by periodontitis, with many inflammatory cells, especially eosinophils. The experimental groups showed an increase in inflammatory cells in both protocols (at 21 days, 61.2±26.13 vs 45.5±45.96, at 28 days, 57.8±26.05 vs 47.5±45.96). At the blood level, the periodontitis group had a higher percentage of neutrophils (15.76±4.68 vs 8.05±0.49; 13.1±8.62 vs 6.95±2.19), basophils (at 21 days 0.14±0.31 vs 0.00 ± 0.00), C-reactive protein (at 28 days 0.005 ± 0.01 vs 0.000 ± 0.00), alkaline phosphatase (at 21 days 108.2±25.33 vs 68.5±13.44), antistreptolysin O (2.63±3.36 vs 1.2±1.41; 5.78±9.32 vs 2.55±0.64), IgA (at 21 days 1.46±0.30 vs 0.90±0.00) and IgG (at 28 days 73.38±8.45 vs 59.5 \pm 0.71). In contrast, they showed lower levels of lymphocytes (82.62 \pm 4.39 vs 90.1 \pm 0.42; 84.94 ± 9.88 vs 91.5 ± 2.55), estradiol (14.58±13.29 vs 24.4 ± 24.89 ; 14.94±12.71 vs 25.3 ± 22.34), progesterone (at 21 days 9.84±3.01 vs 14.15±9.69), iron (at 21 days 419±45.74 vs 478.5±54.45) and urea (42.34±2.38 vs 46±1.41; 42.6±4.34 vs 45.5±0.71). The increased inflammatory parameters in the blood and endometrium showed a clinical correlation with the clinical level of periodontitis. These results seem to demonstrate a consequence of periodontitis on the endometrium and may open new lines of investigation to understand its clinical translation.

Keywords: Periodontitis; Inflammatory Burden; Female Reproductive Organs; Rats



Sexual dimorphism in the prevalence of musculoskeletal disorders among dental students⁺

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Work-related musculoskeletal disorders (MSD) have become a significant concern for healthcare workers, including dentists. Its prevalence among dentists is alarmingly high, with up to 95.8% at risk of developing MSD in their lifetime. This problem can start as early as dental students start their clinical practice, which can have long-term implications for their careers. MSD not only led to sick leave and reduced productivity but also result in early termination of clinical practice. While psychosocial factors may contribute to the prevalence of MSD, the physical burden of clinical work, including incorrect postures and poor body mechanics, is often cited as the primary factor. Furthermore, studies have indicated that female dental students may be more susceptible to developing MSD. This study aimed to compare the prevalence of musculoskeletal disorders between male and female dental students. Participants included fourth and fifth-year students enrolled in clinical practice at Egas Moniz School of Health & Science. Data collection utilized an online form of the Nordic Musculoskeletal Questionnaire (NMQ), known for its reliability and moderate validity in epidemiological studies. Out of 117 contacted students, 85 (72.7%) responded to the questionnaire, and 63 participants were included for analysis. The majority (81%) were female, and 19% were men. Participants had an average age of 22.54 ± 1.51 years, height of 1.67 ± 0.08 m, and weight of 59.95 \pm 11.11 kg. Most reported symptoms in women during the last 12 months were neck (68.6%) and lower back (66.7%) while within men were in lower back (58.3%) and shoulders (41.7%). In the past year, 92.2% of female students and 91.6% of male students reported symptoms in at least one body region. Within a one-week period, the highest occurrence rates were neck (68.6%) and lower back (27.5%) in women and, wrists and hands (75%) and neck (66.7%) in men. In a 7-day span, 58.8% of female students and 50% of male students reported symptoms in one or more regions. Dentistry work involves maintaining static positions for prolonged periods, resulting in excessive pressure on musculoskeletal structures. The study findings corroborate previous studies, demonstrating a higher prevalence of MSD in dental students, particularly in the neck, lower back and shoulders. Sexual dimorphism may contribute to these disparities, with women doubling the men incidence of neck pain. In one-week assessment, symptoms rates were similar between men and women, except for the hands and wrists with men reporting symptoms more frequently (75%) compared to women (15.7%). The underlying causes for these symptoms could be attributed to improper techniques involving sustained contraction of wrist muscles and inadequate ergonomic support, as observed in previous studies. The study underscores the need to address musculoskeletal issues among dental professionals since the student stage, to prevent disability and early retirement associated with these conditions. Tailored interventions addressing the different etiologies and sexual differences among students could effectively reduce the prevalence of MSD. Implementation of preventive strategies is necessary to improve outcomes and promote the well-being of dental students.

Keywords: musculoskeletal disorders; dental students; sexual dimorphism; ergonomy.

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Antimalarial activity of *Tropaeolum majus* L. aqueous extracts and benzyl isothiocyanate ⁺

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Antimalarial drug development remains strongly linked to plant-based pharmaceuticals, as some of the most important therapeutics are based on their chemical scaffolds, such as aminoquinolines (e.g., chloroquine) and endoperoxides, i.e., artemisinin-based drugs. The main causative agent of malaria, Plasmodium falciparum (P. falciparum), has developed resistance to all antimalarial drugs in clinical use, including aminoquinolines and endoperoxides. Furthermore, the development of these antimalarials involves unaffordable environmental and economic costs for most malaria-endemic countries, hence WHO's encouragement of applying natural extracts or plant-based pharmaceuticals based on traditional medicines. Given this, we evaluated the antimalarial activity of Tropaeolum majus L. (T. majus L.) seeds, leaves, and stems aqueous extracts, traditionally applied as antimicrobials, and of benzyl isothiocyanate (BITC), the major biologically active breakdown product from the above-mentioned T. majus L. parts. We used flow cytometry to determine the growth inhibition (GI) percentage of T. majus L. aqueous extracts and BITC against unsynchronized cultures of chloroquine-susceptible P. falciparum 3D7-GFP strain at doses that were within the solvent limits used in antimalarial assays, i.e., 1% and 0.1% of water for the aqueous extracts, and 0.4% and 0.04% of dimethyl sulfoxide (DMSO) for BITC. The correspondent solvents, untreated cultures, and chloroquine (CQ) were included as growth controls. Extracts and/or compounds with at least 70% GI were selected as potential candidates and confirmed by the half-maximal inhibitory concentration (IC50) estimation against unsynchronized cultures of P. falciparum 3D7-GFP and P. falciparum Dd2 (chloroquine-resistant) strains by flow cytometry, and the resistance index (RI) calculated. T. majus L. seed extract had GIs of 38.62 ± 22.89% at 0.132 mg/ml (p=0.0014; Mann-Whitney test vs CQ 10 μ M) and 30.18 ± 13.47% at 0.0132 mg/ml (p=0.0011; Mann-Whitney test vs CQ 1 μ M), the leaf extract displayed GIs of 6.54 \pm 5.32% at 2.51 mg/ml (p=0.0010; Mann-Whitney test vs CQ 10 μ M) and 3.44 \pm 2.67% at 0.251 mg/ml (p<0.001; unpaired t-test vs CQ 1 μ M), and the stem extract only showed a GI of 7.68 \pm 3.15% at 1.32 mg/ml (p=0.0091; Mann-Whitney test vs CQ 10 μ M). BITC presented GIs of 97.13 \pm 0.62% at 3.32 μ M (p=0.1713; unpaired t-test vs CQ 10 μ M) and 14.26 \pm 3.31% at 0.332 μ M (p=0.0110; Mann-Whitney test vs CQ 1 μ M). The aqueous extract solvent did not influence P. falciparum growth, and DMSO did not show meaningful GIs (50.64 ± 3.61% at 0.4% and 4.89 \pm 3.91% at 0.04%). CQ exhibited GIs of 96.57 \pm 0.57% at 10 μ M and 94.71 \pm 2.78% at 1 $\mu M.$ BITC presented IC50 values of 0.217 \pm 0.030 μM and 0.155 \pm 0.005 μM against P. falciparum 3D7-GFP and Dd2 strains, respectively. CQ showed IC50 values of 0.014 \pm 0.002 μ M (p=0.1000; Mann-Whitney test) and 0.181 ± 0.027 μ M (p=0.5512; unpaired t-test with Welch's correction test) against the same P. falciparum strains, respectively. The RIs were 0.71 for BITC and 12.9 for CQ. Aqueous extracts of T. majus L did not display a significant antimalarial activity, probably due to the solvent limits, which limited the concentrations of the extracts. A further extract concentration would be recommended specifically for the seed extract, known for having a higher BITC content than the other plant parts. BITC had a comparable GI to CQ, with similar IC50 values in both strains, and did not show cross-resistance with aminoquinolines as the RI was below 10. BITC presented features that could open new avenues for malaria drug discovery.

Keywords: Antimalarials; Tropaeolum majus L.; Benzyl isothiocyanate; Resistance.



Erythrocyte membrane properties in patients with ALS ⁺

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Erythrocytes have a fundamental role in delivering oxygen to tissues and in binding inflammatory mediators. The morphological and biomechanical properties of the erythrocytes is a new area of study in amyotrophic lateral sclerosis (ALS). To evaluate changes in morphological, biomechanical, and biophysical properties of erythrocytes from ALS patients.We studied blood samples from ALS 55 patients and compared the results with 29 healthy volunteer in order to evaluate the changes in morphology and surface elasticity of erythrocytes. Samples were examined by atomic force microscopy (AFM), and zeta-potential analysis was performed.By AFM imaging, the erythrocyte membrane roughness was smoother in ALS patients (surface roughness 3.67 ± 2.06 nm vs. 6.47 \pm 2.68 nm, for ALS and controls, respectively, p=0.001). These results showed a significant negative correlation with ALSFRS-R. Regarding tip penetration depth into the erythrocyte, this value was significantly lower in ALS patients than in controls ($603.4 \pm 268.0 \text{ nm } vs. 763.7 \pm 246.1 \text{ nm}$, p=0.009, respectively). This value was also negatively correlated to ALSFRS-R (p=0.033) and the respiratory subscore of ALSFRS-R (p=0.014). Zeta-potential was similar between populations (- 12.13 ± 0.24 mV vs. -12.25 ± 0.98 mV, p=0.60), but its change over disease progression correlated with ALSFRS-R decline (p=0.028). Changes in morphological, biomechanical, and biophysical properties of erythrocytes that we disclosed in ALS patients are consistent and their significance require future investigation, in particular they suggest a specific abnormality in the composition of the erythrocyte cell membrane. Moreover, this could imply important hemorrheologic dysfunction increasing the risk of tissue hypoxia. These findings could contribute to dissect the complex interplay between respiratory function, progression rate, and survival in ALS.

Keywords: amyotrophic lateral sclerosis; atomic force microscopy; and zeta-potential; erythrocyte.



Mapping age- and sex-prevalence in adults with Human Immunodeficiency Virus in a subset of the Portuguese population between 2017 and 2021 ⁺

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Since the first diagnosis in 1983, Portugal continuously had one of the highest HIV rates among EU countries. Prevalence remains an important and useful measure of the burden a disease has in the community. Currently, there are 4 HIV affected individuals out of every 1,000 people in Portugal. Age- and sex- specific HIV prevalence estimates are available at the national level, and local estimates are fundamental for planning health services. This is an observational study where we analysed data from 2,322 patients from 2017 to 2021, to determine age- and sex-prevalence in adults with diagnosis of HIV in a tertiary referral centre. According to the 2021 Census by the Portuguese National Statistics Institute, the population covered by this centre was 331,784. For the purpose of evaluating the time trends related to HIV infection, the comparison is usually performed with the penultimate year of a series of diagnosis under analysis, since the value obtained for the most recent year may be affected by a delay in reporting. Inclusion criteria: positive test for HIV, confirmed by Western Blot and/or antibody differentiation immunoassay (Geenius™ HIV 1/2 Confirmatory Assay), in patients \geq 15 years old. According to the latest surveillance data from the Portuguese Health Ministry, 5,625 new cas-es of HIV infection were reported from January 2017 to December 2021 in Portugal. During this 5-year period, 2,322 pos-itive cases were reported in the hospital. In Portugal, the evolution of new cases per year was 1,406 (in 2017), 1,234 (in 2018), 1,182 (in 2019), 870 (in 2020) and 933 (in 2021), which demonstrates a decreasing trend until the year of 2020, with a slight increase in 2021. The number of positive results per year in the hospital were on an upward trend, from 2017 (281 cases) to 2018 (465 cases), reaching a peak in 2019 (634 cases) and dropping from 2020 (560 cases) till 2021 (382 cases). When comparing sex-prevalence, the number of male cases was 1,550 (66.80%) in the hospital, having a similar prevalence to the national cases, which were 4015 (71,37%) male cases. Regarding age-prevalence, the highest numbers of cases reported in the hospital were the groups of 40-49 years old (24.76%; 575) and 30-39 years old (22.18%; 515). In Portugal the group of 30-39 years had the highest number of cases (26.63%; 1,494) followed by the 40-49 years group (21.16%; 1,187). This shows that the hospital's case numbers are in line with the national average. This study of HIV prevalence in a tertiary centre supports the scenario described in the Health Ministry's data and is in accordance with the references described in literature. This research offers a comprehensive and up-to-date understanding of the disease that can be used to provide data to improve the best healthcare strategy to the Portuguese population.

Keywords: HIV; Prevalence; Age; Sex.



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Relationship Between Positive and Adverse Childhood Experiences and Resilience in Adulthood ⁺

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Childhood experiences are important for the individual's development and well-being throughout their life. While adverse childhood experiences (ACEs), such as abuse or neglect, can interfere with the development of resilience, decreasing their levels, positive childhood experiences (PCEs), like having a stable routine, can promote it, enhancing mental and physical health in adulthood. Resilience plays an important role during the child's development and corresponds to the ability to adapt to adversity and overcome difficulties. ACEs can affect resilience through difficulties in establishing emotional bonds, lower self-esteem, and psychopathological symptoms, leading to an impaired capacity for developing resilience. On the other hand, PCEs can contribute to healthy relationships and future social experiences, having a positive effect against adversity through the improvement of resilience and avoidance of adversity reoccurrence later in life. Research suggests that the quality of parental care and support received during childhood can impact the development of resilience and the individual's ability to cope effectively with stressors later in life. The present study aims to analyze the relationship between childhood experiences and resilience in adulthood. Our sample comprised 76 Portuguese adults, 54 women (71.1%) and 22 men (28.9%), with ages between 18 and 70 (M=35.59, SD=15.30). Participants answered online to a Sociodemographic Questionnaire, the Benevolent Childhood Experiences Scale, the Childhood Adverse Experiences Questionnaire, and the Resilience Scale. All participants were granted their consent to participate in this study. The study followed the ethical principles outlined in the Declaration of Helsinki and was approved by the Institutional Review Board of Eqas Moniz School of Health and Science. The results showed that emotional abuse is positively correlated with physical abuse (r=.696, p<.001) and with emotional (r=.546, p<.001) and physical neglect (r=.332, p=.003). Physical abuse is positively correlated to both emotional (r=.416, p<.001) and physical neglect (r=.256, p=.026). There are also positive correlations between emotional and physical neglect (r=.432, p<.001). Negative correlations were found between emotional neglect and self-determination (r=-.343, p=.002), adaptability (r=-.281, p=.014), resilience (r=-.333, p=.003) and PCEs (r=-.600, p<.001). Both physical (r=-.251, p=.029) and emotional abuse (r=-.252, p=.028), and physical neglect (r=-.384, p<.001), are negatively correlated with PCEs. These findings are consistent with existing literature, showing that multiple ACEs (e.g., emotional neglect) are susceptible to co-occurring and are associated with lower levels of PCEs (e.g., supportive family environment) and resilience. This can lead to an individual's impairments in the psychological and social functioning of an individual. On the other hand, studies also reported that the presence of at least 6 types of PCEs could mitigate the ACEs effects, leading to better mental health and the development of resilience through an adequate response to adversity. Assessments of ACEs, resilience, and positive factors (PCEs) will allow a better understanding of the individual's needs and a better adjustment of the support needed for positive functioning and well-being.

Keywords: Childhood Experiences; Resilience; Adults

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HIV-2 resistance testing in Portugal ⁺

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HIV-2 infection is endemic in West Africa and present in some regions of the world, notably Portugal, which has the highest rate of this infection in Europe. Treatment initiation in HIV-2 patients differs from that in HIV-1 patients. In HIV-1, all patients must be treated to achieve an undetectable viral load. Many HIV-2 patients have an undetectable viral load and are therefore not transmitting the infection. Due to this and the fact that there are no specific antiretroviral drugs for HIV-2, the treatment of these patients depends on the CD4+ lymphocyte count, plasma viremia and comorbidities. HIV-2 is treated with HIV-1 antiretrovirals, but there are much fewer therapeutic options for HIV-2 due to natural resistance to some antiretrovirals such as NNRTIs, and resistance selection is much easier in HIV-2 than in HIV-1. Methods: Retrospective analysis of resistance testing to antiretrovirals used in the treatment of HIV-2 infection (nucleoside reverse transcriptase inhibitors (NRTI), protease inhibitors (PI) and integrase inhibitors (INTI)) performed at CHLO, Lisbon, Portugal, between January 2017 and December 2021. Plasma RNA was extracted using the EMAG®. RT-PCR and nested PCR reactions were performed according to an in-house protocol, followed by sequencing by the Sanger method. Identified mutations were interpreted according to the HIV-2 EU algorithmGRADE. Results: 331 resistance tests (RT) were performed; 240 for NRTI/PI and 91 for INTI. Of the 192 sequences successfully amplified for NRTI/PI, 44%, (n=85) were negative and 8%, (n=15) were positive in naïve patients. In treated patients, 19%, (n=36) were negative and 29%, (n=56) were positive. Of the 68 sequences amplified for INTI, 40%, (n=27) were negative and 25%, (n=17) were positive for treated patients. In the naive patients 35%, (n=24) were negative. The most common mutations found were M184V (27%), V111I (19%) and K65R (17%) for NRTIs; I54M and L90M (24%) and I50V (19%) for PIs; N155H and T97A (27%) and E92Q for INTIs. 20% of RT to NRTI/PI and 25% of RT to INTI did not amplify, mainly due to low viral loads. Conclusions: Although not able to amplify all samples, RT are a very useful tool for the detection of HIV-2 resistance both in naive patients and in treatment failures, allowing to change the treatment and avoid the accumulation of resistance that can exhaust the few options available. The most commonly identified resistance mutations have implications for the recommended first-line treatment. Therefore, whenever possible, RT should always be performed before starting treatment.



Development of an antiretroviral resistance test for HIV-2 infection using Next Generation Sequencing (NGS) technology ⁺

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Human immunodeficiency virus type 2 (HIV-2) was identified in 1986 at the Pasteur Institute with the collaboration of Portuguese researchers. It is estimated that there are between 1 and 2 million people living with HIV-2 infection, mainly in West Africa. Portugal is the European country with the highest prevalence of this infection in Europe. Data from Portuguese National Health Institute reports 2102 individuals living with HIV-2 until 31 December 2021. All antiretroviral drugs were developed to treat human immunodeficiency virus type 1 (HIV-1) infection, and their use in the treatment of HIV-2 infection is based on extrapolation. At present, the scientific community is aware that this virus is naturally resistant to several antiretroviral drugs, and that resistance is much easier to develop in HIV-2 than in HIV-1. There are no commercially available tests to monitor HIV-2 infection. Viral load and antiretroviral resistance testing for HIV-2 is of paramount importance for the clinical management of these infections. In the absence of commercial methods, laboratories need to develop tools to monitor people living with HIV-2 infection. Since 2007, our Molecular Biology Laboratory has been performing an *in-house* resistance test, using the Sanger method, to identify HIV-2 antiretroviral resistance. In recent years, new sequencing methods have emerged, that allow the detection of mutations in minority populations with implications for therapeutic choices. To develop and implement an HIV-2 resistance test using NGS, we compared the old Sanger method with the new NGS method to show whether they identify the same mutations and polymorphisms, with a particular focus on those that affect the assignment of resistance to protease (PR), reverse transcriptase (RT) and integrase (IN) inhibitors. Preliminary results are presented here. Methods: 50 plasma samples with viral loads ranging from [65-671,000] copies/ml were tested by both methods. Of these, 24 samples were from individuals on antiretroviral therapy and 26 samples were from naïve individuals. Sanger sequencing was performed using the EMAG® extraction method and amplification was performed according to an in-house protocol. Sequences were interpreted using ChomasPro software v. 2.1.10. For NGS determination, the entire process was automated using VELA Diagnostics, Samtools and GenomeDetective software. The HIV-2 EU algorithm GRADE was used to interpret resistance mutations and polymorphisms in both methods. Of the 50 samples analysed 38 samples were tested by Sanger and 43 by NGS. Of these, 30 samples were compared in both methods. The major mutations detected by both methods were equal as can be confirmed by the Mann-Whitney-Wilcoxon test, with a p-value of 0.317 for the major mutations in the three regions of the pol gene. Based on the data obtained, the NGS method can be used to detect antiretroviral resistance in HIV-2. It identifies resistance mutations as well as the Sanger method with the advantage of detecting minority variants described in the literature but whose data need to be confirmed in this work

Keywords: HIV-2; Mutations; Resistance; NGS; Sanger

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Validation of ultra-high performance liquid chromatography – tandem mass spectrometry method to determine six phosphatidylethanol homologues in whole blood [†]

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Phosphatidylethanol (PEth) is a specific direct alcohol biomarker, with a half-life in blood of approximately 4 days but in some cases up to 12 days, significantly longer than other alcohol biomarkers, such as ethanol. The consumption of alcohol is correlated to the blood concentration of PEth and can be used to distinguish different drinking patterns, such as heavy- and social drinking. In this study, we developed an ultra-high performance liquid chromatography - tandem massspectrometer (UHPLC-MS/MS) method for the quantitative determination of six PEth homologues in whole blood, using previous findings from how to avoid co-elution of PEth and unwanted phospholipids. Chromatographic separation was performed on a C18 column with a mobile phase consisting of 0.025% ammonia aqueous solution and methanol was used. Whole blood samples were prepared by liquid-liquid extraction. The developed UHPLC-MS/MS method for the determination of six PEth homologues was fully validated in whole blood. Each PEth homologues has its own deuterated internal standard, except PEth 18:1/18:1. Inter-assay precision and accuracy were within \leq 18% and \leq 14%, respectively. The extraction recoveries obtained were within 37-51% and no matrix effects were observed for all PEth homologues. The validation showed that UHPLC-MS/MS method is precise, accurate, and sensitive for its purposes and it's a robust and sensitive bioanalytical method for the determinations of the six PEth homologues in whole blood.

Keywords: Alcohol consumption; Phosphatidylethanols; PEth 16:0/18:1; Liquid-liquid extraction; UHPLC-MS/MS; Method Validation

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Language Matters Diabetes Portugal: updated recommendations on the use of language to communicate with and about persons with diabetes [†]

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In 2021, diabetes affected approximately 1 million adults in Portugal (SPD, 2023), leading to a significant burden in terms of morbidity, mortality and costs. The direct cost of diabetes in 2021 was estimated to be over 1400 million euros, which accounted for about 0.8% of the national gross domestic product and approximately 7% of health expenditure (SPD, 2023). Managing diabetes effectively requires an active role of the persons living with the disease, supported by healthcare and other professionals. The way health care professionals communicate can greatly affect persons living with diabetes. Poor communication can cause stigmatization, increase diabetes-related distress, and hamper self-management, which in turn leads to poorer clinical outcomes (Skinner et al, 2020). On the other hand, the effective use of language is expected to reduce stress, increase confidence, and promote better self-management (Lloyd et al, 2018). The movement "Language Matters Diabetes" offers guidance on the use of language in diabetes care in multiple countries around the globe. As part of this movement, in 2022 a Portuguese interprofessional team published recommendations on this matter (Batata et al., 2022), composed of seven principles and three sets of preferred terms: about the persons (10 components), about diabetes (two components) and about diabetes management (11 components). Each component consists of terms to be avoided, terms to be preferred and a rationale. The aim of this work is to report on the latest developments of these recommendations. Revision of the national recommendations, hereby designated as v.1, was engendered by an invitation to contribute with a chapter to the textbook "Manual of Effective Communication in Diabetes", under the auspices of the Portuguese Society of Diabetology. Recommendations were revised through a two-stage approach. Firstly, recommendations were iterated among chapter authors and opportunities for improvement were signaled. Changes in the wording were revised using the lenses of positive writing and rapport. Secondly, professional literary revision offered suggestions for change. The updated version was designated as v.2. Changes between v.1 and v.2 were counted. A total of 14 out of 23 components and all principles underwent changes. Regarding the principles, 12 alterations were made in the wording, considering sentences as the units of analysis. As for the components, changes were implemented in the "rationale" section only, with a total of 18 sentences modified and 4 sentences added. The additional sentences aimed to emphasize the benefits of using preferred terms. A revised version of the national recommendations on preferred language for communication with and about people with diabetes has been published (Batata et al., 2023). Despite being relatively minor in nature, changes are expected to improve clarity and increase engagement with the recommendations. Incorporating these recommendations within a textbook on effective communication enhances its reach and provides a more structured and comprehensive resource for those seeking to enhance their communication skills in diabetes in real world settings.



Keywords: language matters; diabetes; Portugal; recommendations

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Abstract

P.45

Odontogenic Sinusitis Following Dental Implant: Warning Signs ⁺

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The term "odontogenic sinusitis" (ODS) refers to bacterial maxillary sinusitis that may or may not spread to other paranasal sinuses as a result of either concurrent infectious maxillary dental pathology or post-dental treatment problems. The anatomical proximity between the maxillary sinus floor, upper molars, and premolar roots explains this relationship. As a result, maxillary sinusitis may result from periodontal disease or traumatic disturbance of these tooth roots, which could erode the sinus foundation and pierce the periosteum and mucosa. Endodontic disease, periodontitis, oroantral fistulas, and dental procedures involving foreign substances in the maxillary sinus are a few possible dental diseases that might lead to ODS. Early otolaryngology referral is important to prevent complications. We report a clinical case of a 52-year-old male patient who was submitted to a dental implant treatment. In the following days, he complained of frontal headaches, halitosis, and recurrent unilateral purulent rhinorrhea. Imaging showed a fistula in the maxillary sinus floor and severe sinusitis affecting the left fronto-ethmoidal and maxillary sinuses. The patient complaints persisted after antibiotic treatment, and peri-implant granulation tissue formed. Endoscopic surgery with maxillary sinusotomy, fistulectomy, and closure with palate soft tissue graft was performed with complete resolution of the symptoms. Any patient with unilateral sinusitis who has recently undergone dental treatments, especially those involving the upper teeth, or that has a long history of dental issues should be evaluated for odontogenic sinusitis. Effective treatment and complication avoidance depends on early detection, proper imaging, and interdisciplinary management integrating otolaryngology and dentistry.

Keywords: odontogenic sinusitis, dental implant, chronic rhinosinusitis, maxillary sinus, otolaryngology



Local and systemic effects of ligature induced periodontitis and *P. gingivalis* LPS method: a study in rats [†]

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Periodontitis is a multifactorial chronic inflammatory disease, characterised by a marked gingival inflammation with a distinct inflammatory cellular infiltrate and a variety of pro-inflammatory mediators, which is not limited only to the gingival tissues, but also induces systemic changes in the host. As the biological mechanism between periodontitis and systemic effects is still not fully understood and human studies show a high variability, the use of animal models is justified to better understand this pragmatics. In recent decades, several experimental methods have been described to induce periodontitis in animals. Experimental mouse models are good tools to study the mechanisms of periodontal pathogenesis and to test new therapeutic approaches, but there is still a lack of efficacy, complete and detailed description so that they can be easily reproduced. Therefore, the aim of this experimental study is to evaluate the local and systemic effects produced by two different models of in vitro periodontitis induction in Wistar rats, namely by gingival injection of lipopolysaccharide (LPS) from Porphyromonas gingivalis (P. gingivalis) and using the ligadure inducted-periodontitis method. Systemically healthy 7-week-old female Wistar rats (n=28) were housed under stable environmental conditions (temperature 23°C, relative humidity 40%, 12-hours light/dark cycle, and free access to food and water). They were randomly divided into three groups: i) a control group (n=12); ii) a experimental group with 5-0 silk ligature induced-periodontitis (n=5)for 21 days; and iii) another experimental group with periodontitis induced by P. gingivalis LPS injection (n= 11) for 14 days with a total of 6 injections in maxillary second molar lingual side. The inflammatory process in the periodontium and female reproductive organs was evaluated by histological analysis. Serum levels of blood counts, sex hormones and inflammatory parameters were measured. All histological and blood assessments were performed by experienced pathologists who were blinded to sample status. Statistical analysis was performed with a significance level of p-value \leq 0.05. Overall, the ligature-induced periodontitis group had higher lymphocyte (82.62% ± 4.39) and IqG (93.32 \pm 20.7) and white blood cell (2.18% \pm 0.52) and neutrophils (15.76% \pm 4.68) lower than the LPS injection-induced periodontitis and control groups. The ligation-induced periodontitis group was significantly different from the other groups. Inflammation in the uterus, identified in the sample by the ligation technique, shows a possible relationship with alterations in the uterus of female Wistar rats with chronic periodontitis. The ligature-induced periodontitis group shows that the method induced greater periodontal destruction compared to the LPS induction protocol and had a potential impact on systemic inflammatory diseases, both at the blood level and at the inflammatory histological level.

Keywords: Periodontitis, Rat, Ligature induced-periodontitis, Periodontitis induced by injection of *P. gingivalis* LPS.



Employability and dental caries experience⁺

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Currently, dental caries is not just one of the most common oral illnesses across the globe, but it also poses a significant obstacle in public health. To establish preventive measures, it is essential to conduct a risk assessment and identify health factors. The primary goal of this research is to evaluate how employability affects dental caries experience. From January 2016 to March 2020, a crosssectional study was conducted on patients undergoing screening consultation at Clínica Universitária Eqas Moniz - Caparica. The study included an oral examination and a sociodemographic and behavioral questionnaire, as well as radiological evaluation. Employability was classified as either employed or unemployed, while caries experience was categorized as present or absent. The data obtained were subjected to descriptive analysis and logistic regression without indicating the starting point of the original text. Included in the final sample were 9,349 participants aged 18 to 99. In this study, there were 4,980 employed participants (53.3%), 1,083 unemployed participants (11.6%), and 3,286 participants defined as students or retirees (35.2%). In this population, caries experience was 85.5%. Regarding employability, the results showed a significantly lower experience in employed participants when compared to unemployed participants. Individuals who are unemployed display an increased occurrence of tooth decay. Employability should be considered as a relevant health determinant dental health.

Keywords: Dental caries; public health; Risk factor; employability



Emerging Challenges: Prevalence of ESBL- and KPC-Producing *Klebsiella pneumoniae* Strains and the Impact of Biofilm Formation on Antibiotic Resistance [†]

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The rise of *Klebsiella pneumoniae* strains producing extended-spectrum β-lactamase (ESBL) and carbapenemase (KPC) has become a major public health concern. The ability of ESBL- and KPCproducing K. pneumoniae to form biofilms is particularly worrisome due to its potential to facilitate the spread of antibiotic resistance and prolong infections within healthcare settings. In this study, we isolated a total of 45 K. pneumoniae strains from human infections and performed antibiograms for 17 antibiotics, assessed ESBL production using the Etest ESBL PM/PML method, and employed a rapid test to detect KPC carbapenemases. Additionally, we employed PCR to identify the presence of resistance genes. Furthermore, we utilized the microtiter plate method to determine the biofilm production of the isolated strains. Our findings revealed that 73% of the K. pneumoniae strains exhibited multidrug resistance, with the highest resistance rates observed against amoxicillinclavulanic acid, ampicillin, aztreonam, cefotaxime, and trimethoprim-sulfamethoxazole. Conversely, amikacin and tetracycline demonstrated the highest efficacy against these strains. We successfully detected several resistance genes, including bla_{TEM}, bla_{CTX-M}, bla_{SHV}, aadA1, aac(3)-II, tetA, catA, cm/A, gyrA, gyrB, parC, sul1, sul2, sul3, bla_{OXA}, bla_{KPC}, and bla_{PER}. Notably, to our knowledge, our study represents the first report of the blaper gene in K. pneumoniae in Portugal. Regarding biofilm production, our results indicated that 80% of the K. pneumoniae strains were capable of forming biofilms. Interestingly, a significant proportion of ESBL- and KPC-producing isolates exhibited weak biofilm-forming abilities, accounting for 40.0% and 60.0% of the strains, respectively. Notably, we did not find a correlation between stronger biofilm formation and the presence of ESBL and KPC enzymes in the K. pneumoniae isolates. In conclusion, our study underscores the alarming prevalence of ESBL and KPC-producing K. pneumoniae strains and highlights the critical concern of biofilm formation. The high rates of multidrug resistance emphasize the urgent need for effective therapeutic strategies. Understanding the mechanisms underlying biofilm formation and its



association with antibiotic resistance can contribute to the development of targeted interventions against these resilient pathogens.

Keywords: Biofilm; ESBL; KPC; Klebsiella pneumoniae

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Exploring key factors impacting adherence to assistive walking device use in people with Parkinson's disease[†]

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by a progressive loss of motor abilities. As the disease progresses, individuals with PD experience various motor impairments, including gait disturbances, balance instability, and reduced mobility. Assistive walking devices have become vital instruments in addressing the motor difficulties associated with PD and facilitating autonomous walking for individuals with PD. The utilization of these devices presents a significant opportunity to improve the overall quality of life and promote functional independence for people living with PD. However, a significant issue encountered in clinical practice is the suboptimal adherence of patients to these assistive devices. Understanding the reasons behind the lack of adherence to assistive walking devices is crucial for optimizing their utilization. Therefore, this study explores the factors influencing adherence to assistive walking devices in people with PD. We used a qualitative, exploratory, and descriptive approach. The methodology involved conducting one-onone semi-structured interviews with 19 individuals diagnosed with PD. Participants were recruited via social media using convenience sampling. The collected data from the interviews were subjected to thematic analysis. As a result, we identified several factors that influence adherence to walking devices in the study participants that we have grouped into ten themes. 1) Motor symptoms: Motor symptoms, such as gait disturbances and balance issues, affected the patients' ability to use the walking devices effectively. 2) Cognitive abilities: Cognitive impairments, including difficulties with attention and memory, played a role in the patient's adherence to device usage. 3) Restricts use of an upper limb: The user needs to hold the device effectively, restricting the use of the upper limb for functions other than the device. 4) Gait modifications: The walking device can imply a slower gait speed. 5) Self-stigma: Patients' internalized shame or embarrassment about their condition contributed to their reluctance to use walking devices. 6) Social stigma: External societal perceptions and judgments regarding using walking devices influenced patients' adherence, as they felt stigmatized or self-conscious about their reliance on such devices. 7) Feelings of inferiority: Patients reported inadequacy or inferiority when using walking devices. 8) Functional decline: Association between using walking devices and feelings of inferiority, functional decline, and aging. 9) Device prescription: The prescription offers a necessary justification that protects the user from the negative opinions of others. 10) Environmental factors: Uneven surfaces, crowded environments, and weather conditions affected the patients' ability and confidence in using the walking devices. By identifying these influential themes, this research provides valuable insights into the complexity of factors that impact adherence to walking device usage among the study participants. This knowledge can guide the development of targeted interventions and support strategies to enhance adherence and improve the overall management of the disease.

Keywords: Parkinson's disease; patient compliance; walking aids; walking device; mobility limitation; accidental falls.



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What is the incidence of oral lesions at Clínica Dentária Universitária Egas Moniz? A retrospective study of the last 4 years – Preliminary results [†]

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The oral cavity can be affected by a wide range of lesions of different origins and characteristics, which can have a negative impact on people's quality of life. Cancer is the second leading cause of death in Portugal and there has been little improvement in cancer mortality over the last decade. Thus, it is very important for clinicians to understand the distribution of lesions more commonly found in the oral cavity in order to better determine the most appropriate diagnostic and/or therapeutic approach. Therefore, the aim of this study was to evaluate the frequency of oral lesions undergoing biopsy at the Clínica Dentária Universitária Egas Moniz (CDUEM) in the years 2019, 2020, 2021 and 2022, according to location, age, and sex. A retrospective study was carried out using the CDUEM database of anatomopathological reports. Information on the sex and age of the patients and the location of the lesions were analysed. Of the 131 biopsies, 57 (43.51%) were from males and 74 (56.49%) were from females, ranging in age from 10 to 90 years, with a mean age of 54.15 ± 18.16 years. Buccal mucosa was the most commonly biopsied area (25.95%), followed by the lip (13.74%). Benign lesions were the most common (89.39%), followed by potentially malignant lesions (8.33%) and malignant lesions (2.27%). Fibrous hyperplasia was the most common diagnosis in the total sample (31.30%), followed by radicular odontogenic cyst (6.11%). In terms of malignant lesions, squamous cell carcinoma (0.76%) and lymphoid neoplasm (0.76%) were detected. The present study not only provides useful information on the incidence and distribution of oral lesions, but also draws attention to early diagnosis, which is essential to save patients' lives and minimise the negative impact on their quality of life.

Keywords: oral cancer; biopsies; oral medicine; oral lesions; anatomopathology; oral health; public health; early diagnosis; epidemiology

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Higher cumulative doses of doxorubicin as a Chemo Brain trigger $^{\rm t}$

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Chemobrain, the most common neurological consequence following chemotherapy, and anxiety disorders are widely common complications of cancer therapy with doxorubicin (DOX). They dramatically deteriorate patients' quality of life by interfering with the control of different cognitive domains, changing various aspects of memory, emotion, and executive function. Despite the large number of studies addressing chemobrain physiological consequences, is not yet known the individual contribution of DOX treatment and cancer by themselves on the observed effects, since these adverse effects may be caused by cancer itself, cancer therapy, or both. Thus, to clarify DOX action on behavioral disturbances upon cumulative treatment, in this preliminary study on a healthy animal model, we determined the extension of doxorubicin effects due to various therapeutic doses on cognitive decline, anxiety, locomotor activity, as well as neuroinflammation. For that, adult, normal weight, female Wistar rats were DOX-treated for 4 weeks: LDOX (n=8; low dose, 2mg/kg/week), IDOX (n=8; intermediate dose, 4mg/kg/week), HDOX (n=8; high dose, 5mg/kg/week) and control (n=5; NaCl 0,9% saline solution; 1ml/Kg/week). Behavioral tests of anxiety, locomotion, and exploration - open field test (OFT), elevated plus maze (EPM), and cognition performance (Y-maze) were accomplished. GFAP, Iba-1, and Neu-N expression in the hippocampal dentate gyrus were assessed to determine the brain inflammatory state. Our results showed that the three DOX dosages induced anxiety-like behavior and locomotor activity impairments. Moreover, DOX treatment, independent of the dosage, induced hypolocomotion which was accompanied by hypoactivity. In addition, IDOX and HDOX dosage caused short-term memory. Behavioral deficits were corroborated by reactive astrogliosis (HDOX: 47±13 vs. CTL: 15±2) and microgliosis (IDOX: 5 ± 1 ; HDOX: 4 ± 1 vs. CTL: 2 ± 1), due to neuroinflammation in the hippocampus. These findings suggest that the DOX effects on behavioral function are different according to the cumulative dosage, with the highest dosage causing the most deleterious effects on the evaluated behavioral parameters which positively correlate with the hippocampus neuro-inflammatory state. Although further studies are needed to assess the effect of cancer on the etiology of behavioral impairment, the present data support the hypothesis that neuro-inflammatory status induced by higher cumulative dosages of DOX can be part of the etiology of cognitive symptomatology in cancer patients treated with DOX.

Keywords: Doxorubicin; Anxiety; locomotion; chemotherapy-induced cognitive impairment, cognitive dysfunction; astroglial activation; reactive microglial; neuroinflammation

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Applying the Project Management Methodology (PM²) to an interdisciplinary R&D project ⁺

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The VA|PREVENTION project aims to evaluate the effectiveness, implementation, and costeffectiveness of a novel person-centred digital intervention to prevent type 2 diabetes in high-risk adults. The project consortium consists of 5 academic partners, supported by national funds with a total budget of EUR 250 K. The PM² methodology, developed by the European Commission, was selected for waterfall project management, as it provides a structured approach based on acclaimed frameworks and offers a range of open access resources (European Commission, 2021). There is a scarcity of papers reporting the use of PM² in R&D projects. This paper contributes to address this gap by reporting on the application of PM^2 in the planning phase, to support the development of the Project Handbook. PM² methodology v3.0.1 was applied by the project coordinator (PC), the project manager (PM) and a project manager assistant (PMA). Both the PC and the PM are researchers with training and/or experience with the PMBOK framework whilst the PMA is a student doing a capacity building internship. The Project Handbook documents the approach for implementing the project goals, key controlling processes, policies and rules, plus the overall management approach. The PC, PM and PMA developed the Project Handbook based on the PM² templates, through loops of discussion for deciding on aspects such as tailoring, i.e. which artefacts templates would be used. The grant application was a key input for the development process. Involvement of the remaining team members was ensured through a presentation of the draft version in the planning kick-off meeting followed by a period for comments and feedback on the Handbook. The VA|PREVENTION Project Handbook v.1 is composed of the same 6 sections as the PM² template (about, overview, approach, processes, progress measurement, roles and responsibilities). Sixteen artefacts templates were excluded; reasons were documented in the Handbook. For example, risk and issue management plans were replaced by descriptions in the Handbook coupled with the use of available artefacts (risk and issue logs). A total of nine additional artefacts was used; customization was kept to minimum. The Handbook and associated artefacts were stored in the project shared repository, to ensure accessibility. The application of PM² methodology to an interdisciplinary R&D project streamlined the development of the Project Handbook and associated artefacts in relation to prior projects. The project type, goal, needs and resources are key considerations for tailoring this methodology. Nonetheless, the process demands skilled human resources and a time commitment that may not be align with smaller projects. Worked examples of Handbooks specifically for R&D projects can further enhance efficiency. Furthermore, it may be helpful to avoid falling into the trap of perfectionism and instead embracing the concept of a living document, which can be update based on emerging experiences and regular reviews of the methodology and artefacts.

Keywords: project management methodology; R&D project; VA|PREVENTION Project Handbook, European Commission

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X-Ray Vision: Self-Prevention of Oral Health knowledge among adolescents undergoing orthodontic treatment ⁺

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Oral Health is crucial particularly in adolescents' patients. Because of the physical and mental transformations happening in this phase. Therefore, habits acquired in this phase, tend to have future repercussions on the self-esteem, and health behavior of the individual. However, studies showed that in this period, behaviors that are favorable to the oral health appear to decrease since oral health is considered as less important in their perspective. Measures such as Self-Prevention and Self-Promotion does not seem as effectible as they should be. This line of study aims to identify the mental representation of the body, namely, the inner oral cavity. In the present study, we seek the perception of adolescents on this topic with the mere objective to contribute to the creation of innovative oral health strategies and Health instruments. This study has assessed the dental hygiene habits and knowledge regarding oral health using a questionnaire that was given to the participants. The sample consisted in 200 subjects aged between 10 and 24 years old in consultation at Egas Moniz University Clinic. They were first asked to draw the interior of their oral cavity before and after undergoing orthodontic treatment. Then, they were invited to answer a questionnaire concerning oral health behaviors and knowledge. Finally, the patients were given information to help them maintain and improve their self-care. The data was then analyzed using various tools, among them, a content analysis grid, created especially for this study, that was made of analytical categories and subcategories. The data obtained was also analyzed using the statistical software IBM SPSS 28. While adolescents are at risk of developing dental health problems due to their oral health habits but also their knowledge and motivation, the results showed that there's a significative lack in the knowledge of adolescents, especially in terms of frequency of brushing, duration, and technique of brushing. Only 67 patients (33.5%) revealed to brush the intern surface of the tooth, and most of the sample (62%) admitted brushing their teeth for 2 minutes approximately. Although, 78.5% of the adolescents, through the orthodontic consultations reported to have access to information regarding their health, results showed that they didn't use them properly. Regarding the results obtained from the drawings content analysis, they indicate that there's decrease area of knowledge surrounding the interior of the oral cavity. Although, the subjects were asked to draw the interior of the oral cavity, 55.5% represented a Broad Smile in M1 (before) and 60.5% in M2 (after the orthodontic treatment) supported by an Extra-oral view. To conclude, adolescents need to receive a better education on Oral hygiene care in order to enable the self-prevention and self-promotion and hence capacitate them. Information should be given regularly as they showed efficacy on the short-term and should be continually reminded. To this end, the shortage of knowledge proves the need to invest in Oral Health Education.

Keywords: Oral Health; Health Education; Adolescents; Self-Prevention; Health Promotion



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Validation of the Portuguese version of the Oral Frailty Index-8 ⁺

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As life expectancy continues to increase, the world is experiencing an unprecedented rise in the aging population, and with it a series of unique challenges for health and society, including the need for support for the growing needs of the elderly population and management of the social and economic impacts associated with it. The concept of oral frailty has gained scientific and clinical relevance in recent years, and early identification and intervention may prevent its onset. The Oral Frailty Index-8 (OFI-8) has been developed for surveying community-dwelling older adults at risk of oral frailty. This research aims to explore the psychometric validity of OFI-8 in the Portuguese population, named OFI-8-PT, which can serve as a reference for future studies related to longevity and oral function. The present study included two main phases involving patients with 60 years old or older, Portuguese speakers and those who consented to participate in the study. Researchers first translated and cross-culturally adapted the original questionnaire to make it suitable for Portuguese native people. Then, the translated tool was assessed for its psychometric validation, which consisted of test-retest reliability, internal consistency, construct validity, and gender invariance measurement. A total of 158 elderly individuals participated in the baseline survey, consisting of almost an equal number of male (79) and female (80) participants. The OFI-8-PT demonstrated good reliability (Cronbach's alpha = 0.95) and construct validity (GFI = 0.96; CFI = 0.85; and, RMSEA = 0.05; 90% CI 0.00–0.09). The study found gender invariance, suggesting that the OFI-8-PT is equally effective for both males and females and the test-retest reliability of the OFI-8-PT was good, indicating consistent results over time. Overall, the study provides valuable insights into the psychometric validity of the OFI-8-PT questionnaire in the Portuguese population under study. OFI-8-PT allows identifying individuals at risk of oral fragility and preventing it.

Keywords: Oral Frailty; Elderly; Oral health

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Unusual mortality event (UME) of wild Atlantic puffins (*Fratercula arctica*) in the Portuguese coast – preliminary investigations ⁺

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Systematic surveillance of wildlife mortality events renders vital information regarding populational trends and specific threats for individual fitness. While informative, wildlife mortality surveillance can be challenging. Atlantic puffins (Fratercula arctica) inhabit the North Atlantic and are only present in Continental Portugal coasts in the Winter and during their migration towards southern areas. Between January and February of 2023, an unusual mortality event (UME) of Atlantic puffins was observed in several areas of Continental Portugal. Dedicated surveys in areas of higher observed mortality were conducted in the monitoring framework of the Lisbon and Tagus Valley Stranding Network (RALVT). Additionally, informal reports of mortality from volunteer observers were also collected and geographical and temporal distribution of mortality cases was compiled. Eleven deceased animals were collected during beach surveys, based on decomposition status, and individual post-mortem examination was conducted. Direct contact smear was performed to the middle section of the kidney of each individual due to reports of parasitic infection in previously described cases. Furthermore, histopathological examination of kidneys was conducted for each bird to evaluate associated lesions. Beach surveys conducted by RALVT resulted in the detection of 47 dead animals on beaches of Lourinhã and Torres Vedras municipalities, with higher prevalence in the latter (93.6%, n=44). Additional reports accounted for 315 dead animals ranging between Torres Vedras and Peniche municipalities. Post-mortem investigations revealed a predominance of females in the investigated sample (72.7%, n=8). Age class distribution was homogenous. Severe emaciation was evident in every examined individual, with no macroscopical pathological alterations noticed. From the 11 examined animals, one individual presented trematode-like structures in the renal tissue. A complete trematode parasite was observed presenting two suckers in a branch of the ureter, that presented ectasia. Histologically, the kidney presented autolytic changes. The structures compatible with trematode parasites were filled with trematode type eggs and, in some, it was possible to identify oral and/or ventral suckers. Investigations of the UME of Atlantic puffins in Continental Portugal revealed a general emaciation pattern in the population compatible with high energetic costs associated with the migratory process without a proper ingestion of prey. Sea current shifts resulting in differences of prey distribution and consequent non-overlapping of prey and predators can explain the observed phenomenon and can be expected to increase in frequency with climactic alterations. Detection of parasitic stages in internal organs raises additional concern regarding onset of mortality. Morphological resemblance with parasites of the genus Renicola, previously detected in renal structures of piscivorous birds, suggests a similar parasitic involvement. Direction of causality between mortality and parasitic infection is difficult to determine in small samples and further investigation is needed to clarify the possible link. Future genetical evaluation of the parasites, as well as a continuous monitoring effort during the next migration season, will help shed light in this unusual mortality phenomenon.

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Keywords: puffins; unusual mortality event; trends; kidney trematode-like parasite.

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Abstract

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Histological Observations of Peri-implantitis ⁺

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Periodontitis is characterised by chronic microbial inflammation modified by the host, resulting in loss of periodontal attachment. Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia and Treponema denticola are among the aetiological microbial agents of periodontitis. Periimplantitis is defined as a pathological condition that occurs in the tissues around dental implants and is characterised by inflammation of the peri-implant mucosa and progressive loss of supporting bone. Although the periodontium and peri-implant supporting structures share similar histological and clinical characteristics, in the light of current knowledge, the differences between periodontitis and peri-implantitis are not yet well understood. Therefore, the present study aims to describe the histological observations of peri-implantitis and compare them with the characteristics of periodontitis. Gingival samples of peri-implantitis were collected. Half of the samples will be prepared for light microscopy observation using standard H+E methods. The other half will be fixed in glutaraldehyde and cacodylate buffer, prepared semi-thin cuts and observed by electronic microscopy study. Areas with inflammatory infiltrates were observed. In the peri-implantitis lesions, plasmocytes, lymphocytes and polymorphonuclear cells in an apparent state of necrosis were identified, as well as modified macrophages occasionally organized in a palisade. Some studies have reported that peri-implantitis lesions have a higher number of dispersed macrophages in the soft tissue compared to periodontitis. Ethical approval of the study protocol was obtained from the Egas Moniz Ethics Committee. Our observations show a high number of macrophages and some cells with nuclear degradation that are difficult to identify. These observations support and extend previous histological findings in peri-implantitis. The preliminary observations of this study show a large number of cells in the process of necrosis, with significant macrophage presence. It is imperative to continue the observations and increase the number of samples in order to obtain comparative results. The observations will be supplemented by immunomarker studies.

Keywords: peri-implantitis; periodontitis; plasmocytes; lymphocytes; polymorphonuclear cells; macrophages; histology.



Improving Shoulder Function in Breast Cancer Survivors: The Impact of an Exercise Program ⁺

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A significant number of breast cancer survivors exhibit alterations in scapula-humeral rhythm. The gleno-humeral joint is highly mobile, and its stability heavily relies on neuromuscular control. The assessment and treatment for its normalization should be integrated into the physiotherapy clinical settings for shoulder joint disfunctions. In other populations, exercise therapy and scapular stabilization exercises were found to be an effective approach for controlling pain, promote normal motor control and decreasing disability. The aim of this study was to evaluate the effects of a dynamic stability exercise program on muscle activation patterns, scapular position, pain, and functionality, in women who have undergone surgical treatment for breast cancer. This is a quantitative, quasiexperimental study with a longitudinal design. The sample consisted of 24 women: the experimental group (EG=12) underwent conventional physiotherapy along with a dynamic stability exercise program for the shoulder joint complex, while the control group (CG=12) received the forementioned conventional physiotherapy. Both groups were reassessed at the end of an 8-week intervention. In the initial assessment, we found significant differences in the activation of upper trapezius muscle between the affected and unaffected side (p<0.05) in both groups. The EG showed significant improvements in the activation of the anterior deltoid, symmetry of scapular position, increased shoulder flexion and abduction ranges of motion, and improved functionality. The CG showed significant improvements only in shoulder flexion and abduction. The dynamic stability exercise program for scapula-thoracic region has an immediate beneficial effect in reducing pain, improving joint ranges of motion, enhancing symmetry in scapular position, and promoting functionality. However, further studies with larger samples and more detailed kinematic analysis are needed.

Keywords: physiotherapy, electromyography, scapulohumeral rhythm



Influence of Social Conditions on Memory Decline, Risk of Dementia and Depression, and Social Involvement in Island Old People[†]

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Although the disease prevalence in older people increased over time, little is known about this trend regarding cognitive function and dementia, particularly in institutionalised individuals. A study with institutionalised older adults showed, however, that helping to enhance or maintain the quality of their social network allowed improving cognitive functioning, decreasing depression, and promoting quality of life. Stimulation activities, both mental and social ones, are known to be protective against dementia risk, as well as preventing cognitive decline. Furthermore, evidence suggests that social involvement protects against developing depressive symptoms in older people. The present study aimed to verify the influence of social condition - living in nursing homes or attending social centres - on memory decline, on risk of dementia and of depression, and social involvement of older adults living on Terceira Island - Azores. The Faculty of Psychology-University of Lisbon's ethics committee approved the project. Thirty-eight older adults participated in the study: 18 (9 women) residing in nursing homes aged 67 to 95, and 20 women attending two social centres aged 65 to 83. Participants were recruited from their Institutions (nursing homes and social centres) or by word of mouth. They voluntarily participated in the study, having provided a written informed consent. Several assessment scales were administered in three individual sessions at the institution's facilities: the Weschler Memory Scale - 3rd Edition, the Dementia Rating Scale - 2, the Geriatric Depression Scale - 15, and the Social Involvement Scale. Two hypotheses were raised: 1 institutionalised older people would differ significantly from the ones participating in social centres, being more prone to memory decline, risk of dementia, depressive symptoms, and having a lower level of social involvement; 2 - social condition would predict these four variables. A multivariate analysis of variance carried out on participants' performance in the studied variables revealed statistically significant differences between the two social conditions. As expected, the older adults participating in social centres had more positive outcomes than the institutionalised older people regarding memory decline [F(1,36) = 24,43, p < .001], risk of dementia [F(1,36) = 27.38, p < .001].001], depressive symptoms [F(1,36) = 6,21, p < .05], and level of social involvement [F(1,36) =27,90, p < .001]. Furthermore, hierarchical regression analyses showed that (i) social condition predicted memory decline (β = .48, p <.05) and risk of dementia (β = .34, p <.05), (ii) the principal factor associated with depression was social involvement (β = .74, p <.001), and (iii) the main factors related to social involvement were age ($\beta = .56$, p < .001) and gender ($\beta = .32$, p < .05). This set of results broadly supports the hypotheses raised and is consistent with the findings of previous studies. Thus, some implications emerge in view of positively benefiting the ageing of institutionalised older people. They suggest the necessity of encouraging activities that promote social involvement, developing mental and social activities that stimulate cognitive functioning and protect against loneliness and risks of depression while respecting the diversity and idiosyncrasy of the ageing process in each individual.

Keywords: ageing, social conditions, memory decline, risk of dementia, risk of depression, social involvement.

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Exploring the Influence of Physical Activity on Lymphedema Development in Female Breast Cancer Survivors [†]

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In recent years, there has been a notable rise in the number of breast cancer survivors, highlighting the advancements in treatment and care. However, the presence of cancer treatment side effects, such as lymphedema (LE), significantly affects the function, active participation, and overall quality of life for these individuals. It is imperative, therefore, to gain a deeper understanding of the most effective strategies for preventing and treating LE. Preventing LE involves promoting active and healthy lifestyles by instilling behavioral changes and addressing modifiable risk factors. Therefore, this study aimed to characterize the physical activity (PA) levels of Portuguese breast cancer survivors. A cross-sectional analytical study was conducted. Women who survived breast cancer between 1 and 5 years after surgery were selected. A characterization questionnaire was performed, upper limb lymphedema volume was measured with tape measures and PA levels were assessed with International Physical Activity guestionnaire (IPAQ-SF). A group of 20 women with a mean age 61 ± 8 years and an average of 39 months after breast surgery have participated in the study. From those, 80% were overweight, of which 35% were obese. There was an average volume of 2100cm³ in the affected upper limb and an average of 2013cm³ in the unaffected side. The difference between the two limbs presented an average of 87.3cm³. So far, none of the participants showed a significant difference between limbs (greater than 10% difference). Despite this, 45% of participants have subclinical LE (difference between both upper limbs of 5 to 10%). No significant associations were found between the LE and PA (rs=0.231; p=0.3) nor between LE and the number of hours sitting (rs=0.291, p=0.213). However, a strong positive correlation was found between Body Mass Index (BMI) and limb volume difference (rs=0.583; p=0.007). The study does not provide evidence of a direct association between physical activity (PA) and lymphedema (LE). However, indicate a correlation between body mass index (BMI) and lymphedema, and higher BMI is identified as a risk factor for the development of lymphedema. Moreover, engaging in physical activity can contribute to the control and management of BMI, revealing the influence of PA in BMI. Consequently, PA indirectly serves as a preventive strategy against lymphedema by helping to control BMI. By incorporating PA as part of a comprehensive approach to managing BMI, the risk of developing lymphedema may be reduced. In summary, the observations suggest that while PA may not directly prevent lymphedema, it can play an important role in indirectly preventing its development by helping to control BMI.

Keywords: physical activity; breast cancer survivors; lymphedema



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My Tooth is Ill: (Un)Healthy Tooth Profiles among Children (Phase I e II)⁺

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Dental caries has been considered a public health problem insofar as it constitutes a source of discomfort, pain and aesthetics with implications for the general well-being of the individual. It is essential to understand, analyze and characterize the mental representation of children about this concept, in order to contribute to the (re)conceptualization of Oral Health Education in terms of the etiology of caries. Two studies (Phase I and II) were carried out with the aim of characterizing the mental representation of an (Un) Healthy Tooth, associated with the concept of Dental Caries mentally internalized by children. This oral communication presents the results of an exploratory study divided into two distinct phases (Phase I: n=880/4-9 years; Phase II: n=812/6-12 years) that involved a total of 1692 children, recruited at the Schools of the Municipality of Lisbon and at Egas Moniz University Clinic. Data was collected during two moments: M1, where the child was asked to draw a Healthy Tooth on a sheet of paper and M2, where the child was asked to draw an Unhealthy Tooth on another sheet using only a pencil of graphite, totalling 3384 drawings. Subsequently, the child was asked to answer to an open-ended questionnaire, composed by three different questions, with the aim to evaluate the mental representation of the concept of: a) Dental Decay, b) Healthy Tooth and c) Unhealthy Tooth. The data collected was then analysed, using a content analysis grid, especially elaborated for this study, and composed by analytical categories and subcategories. In this work, the data from the content analysis of the drawings, was made by 4- to 12-year-old children. The symbolism of the teeth that were drawn tends to increase with chronological age between 4-6 years old, denoting a higher frequency of unrealistic teeth drawn from Phase I (83.3%) to Phase II (18.7%) and at the same time from 10 years and onwards there is a marked decrease in this pictorial (Un)Realism. Most children in both Phase I and Phase II associate a healthy tooth with a clean tooth with a smooth surface. In contrast to the mental representation of the diseased tooth in both phases, in which the caries category is represented at the level of the categories: stains, fractures and cavitation. This exploratory study aims to contribute to the creation of playingpedagogical instruments in the child's clinical setting. Analysis of the pictorial contents of the drawings made by the participating children denotes significant discrepancies, inherent to the illustration profiles of healthy tooth versus unhealthy tooth. Such discrepancies bear implications at the level of Oral Health Promotion and Prevention, suggesting the need to create ludic-pedagogic instruments for oral health education in very early development stages.

Keywords: Oral Health Education; Drawing; Dental Decay; Healthy Tooth, Unhealty Tooth; Mental Representation



Oral Health in nursing home residents preliminary results of an exploratory crosssectional pilot study[†]

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According to the World Health Organisation (WHO), dementia is a group of disorders that affect memory, thinking, and the ability to carry out daily activities. It is the leading cause of disability, dependency, and mortality, particularly in the ageing population. Patients with dementia are more likely to have poor oral health and nowadays, a good oral health is now a critical factor in the overall health and well-being of older adults. This study was conducted to assess the oral status of nursing home residents diagnosed with dementia in the Lisbon region, Portugal. In this cross-sectional observational pilot study, oral and dental status were evaluated by determining the Decayed-Missing-Filled-Tooth (DMFT) index, frequency of oral hygiene, presence of erosion lesions or mucosal lesions, presence of dentures and Short Xerostomia Inventory application. This study aims to be a starting point for a broad analysis of this population and to further determine potential associations between oral status and anamnestic factors and dementia data.

Keywords: Oral health, Nursing home, Dementia


Identification and antifungal patterns of cryptic species of Candida isolated from ICU patients ⁺

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Candida species are emerging as a major cause of life-threatening infections in immunocompromised patients. There has been increasing recognition of the cryptic Candida species, which are organisms that are morphologically indistinguishable but which can be differentiated by molecular methods. These organisms have been known to show a higher minimal inhibitory concentration for most of the antifungal agents in vitro. Therefore, correct identification of these cryptic species is very important to administer a proper antifungal agent. In this study, we wish to identify and characterize antifungal patterns of Candida cryptic species from Intensive Care Units (ICU) clinical samples. This multicenter prospective study was conducted from January 2020 to December 2022. Axillar/inguinal swab patient samples were collected at admission and on 5th and 8th day of ICU stay from 674 patients. This investigation has been approved by the Institutional Ethical Board of all institutions enrolled. All samples positive for Candida species were included in this study. Identification of the isolates was done using phenotypic methods and by Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). For the analysis of cryptic species, the isolates underwent polymerase chain reaction (PCR). In vitro, antifungal susceptibility tests were performed for fluconazole, voriconazole, amphotericin B, and anidulafungin, according to the concentration gradient Etest® strip technique. A total of 988 samples were received from ICUs during the study period, of which 355 Candida spp. isolates, 185 were identified as C. albicans complex, 112 as *C. parapsilosis* complex, 36 as *C. glabrata* complex, 15 as *C. tropicalis*, 4 as *C. lusitaniae* and 3 as *C. guilliermondii*. The MALDI-TOF (Vitek MS database) and PCR analysis for the identification of cryptic species revealed that three isolates (0.8%) were, actually, cryptic, two were C. orthopsilosis and one was C. metapsilosis. All cryptic isolates were susceptible to the four antifungals tested. Currently, local available data on cryptic Candida species is very limited. We did not find significant differences in susceptibility pattern neither in azole resistance between sensu stricto and cryptic species. Based on these results, and although considering the limitation that the percentage of cryptic species isolated in this cohort is very low, it seems that the identification of the Candida isolates to its cryptic species is not a critical step in antifungal prescription.

Keywords: Candida spp.; ICU; cryptic species; antifungal; resistance.

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The impact of Mechanical Stimulus on the Temporomandibular Joint's Cartilage: a systematic review and meta-analysis [†]

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The temporomandibular joint (TMJ)'s fibrocartilage develops in response to the local mechanical environment as a load-absorbing structure, essential for maintaining chondrocyte proliferation and extracellular matrix production. Several animal studies analyze this tissue's response to different types of mechanical stimuli. This systematic review and meta-analysis intend to evaluate in vivo the morphological and cellular changes in TMJ's fibrocartilage in response to different modalities and intensities of mechanical loading stimulus. PubMed, SCOPUS, and B-On databases were searched for studies published between 2011 and December 2021. Hand-searching through citations was also performed. Only experimental in vivo studies that provided knowledge about the effect of clinically applicable mechanical stimuli that alter the magnitude of TMJ, published in the previous ten years, were included. These were grouped into four categories, according to the type of stimulus: compressive force loading, anterior mandibular displacement, increased occlusal vertical dimension, and diet hardness. Random effects meta-analysis was performed for the effect of compressive force loading on the cartilage thickness. 25 articles were considered for the qualitative synthesis and 6 articles were included in the quantitative synthesis. The results revealed that different dietary consistencies promoted differences in the fibrocartilage thickness and histological content. Mandibular advancement was also associated with metabolic changes. The increase in vertical dimension revealed contradictory results. The compressive force loading reduced by 0.49µm/g.day the fibrocartilage thickness. The rats' initial age was a significant factor in this wear (p < 0.05). In conclusion, mechanical stimulus significantly affected the temporomandibular joint's fibrocartilage, showing not only morphological alterations but also changes in its cellular and metabolic activity, which seem to be related to the period and intensity of the stimulus.

Keywords: Temporomandibular joint, Fibrocartilage, Mechanical stimulus, in vivo.



Evaluation of Erosive Wear prevalence using different evaluation systems ⁺

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Dental Erosion is the chemical wear not caused by acids of bacterial origin, in the absence of dental plaque, as a result of intrinsic or extrinsic acids. Erosion can positively affect abrasion and attrition, enhancing its wear by the initial demineralization of tooth substrate. Intrinsic acids such as gastric juice in patients with gastroesophageal reflux disease and extrinsic acids from diet and occupational exposure to acids are considered aetiological factors to dental erosion. To date, very few data is available regarding Erosive Wear (EW) prevalence. The aim of this study is to assess the prevalence of Erosive wear in the Screening and Urgency appointment in Egas Moniz Dental Clinic (EMDC) using two different evaluation systems. If informed consent properly signed, data from EMDC patients was collected between 2016 to 2022. Two different indexes were used to record erosive wear, between 2016-2019 a dichotomic question was used (present or not present), from 2021 to 2022 a system developed by the World Health Organization was used. Data was organized into evidence tables and analyzed using descriptive statistics from SPSS Statistics (version 28.0 for Mac, IBM) A total of 8773 patients were included, in which 176 patients presented erosion lesions. Prevalence of erosive wear between 2016-2019 and 2021-2022 was 1,5% and 3,5% respectively. The prevalence of Erosive wear seems to have increased from 2016/2019 to 2021/2022 (1,5% to 3,5%) which may be due to a real increase in prevalence or to a better diagnostic tool to identify erosive wear lesions. The new classification system allowed to record the presence of Erosive wear concomitant with other tooth wear lesion (293 patients, 13,8%), which was not possible in the period 2016-2019. Also, with the WHO classification system it was possible to identify the lesions involving only enamel as the most prevalent type of erosion

Keywords: Erosive Wear; Tooth Wear; Evaluation system



Evaluation of Glycemia, Glycosylated Hemoglobin and Body Mass Index (BMI) in adolescents ⁺

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Diabetes is a disease with a strong incidence in the population, several studies prove that lifestyle and diet are two of the factors that greatly contribute to the onset of type 2 diabetes in adults. Early screening is crucial in combating and treating this pathology, and the importance of evaluating these parameters is often overlooked, especially in the younger population. This investigation has as main objective to perform screening test of Glycemia, Glycosylated Hemoglobin and Body Mass Index (BMI) in students of secondary schools and as secondary objectives to describe and frame the levels evaluated, to detect potential cases of pre-diabetes or of students already with the pathology and who were unaware of it, clarify and refer to a medical consultation to confirm the pathology. The screening tests were carried out in 234 students of the 10th, 11th and 12th grades of the Secondary Schools of Moita and Ramada, through a level I study, descriptive, quantitative and transversal with a qualitative and quantitative methodology. The data collected was confidential and recorded in tables and later treated with SPSS (Statistical Package for the Social Sciences) version 28.0 and Excel software. 5% (11 cases) had altered glycemia levels with 4 elevated cases of HbA1c. The vast majority of students of male and female students (69% of female students and 73% of male students) have a normal" BMI value. There is also a considerable proportion of students who are Overweight" (21% of females and 16% of males), and "Obese" (9% of males). In cases where the results indicated pre-diabetes or diabetes, this information was given to the students' responsible in a closed letter or via email.

Keywords: Blood glucose; Glycosylated hemoglobin; Students; BMI; Diabetes

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Establishment of Reference Intervals for Gla Rich Protein Serum levels and variations in a Peritoneal Dialysis Population ⁺

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Cardiovascular disease (CVD) is the major cause of morbidity and mortality in the developing world and the major cause of death in chronic kidney disease (CKD). Vascular calcification (VC) and inflammation play key roles in both diseases and are involved in a complex/interconnected cycle powering pathophysiologic mechanisms leading to disease progression. New diagnostic/prognostic tools are required for early detection of VC and disease monitoring allowing early medical care intervention and increase treatment efficacy. Gla rich protein (GRP) is a circulating vitamin Kdependent protein with anti-inflammatory and vascular calcification inhibitory properties, recently proposed of potential clinical utility as an early marker of vascular damage in CKD. Using a validated sandwich ELISA kit for total GRP conformations (tGRP), we aimed to establish the reference interval levels for serum tGRP in a healthy adult Caucasian population, the variations in peritoneal dialysis (PD) patients and the associations with cardiovascular risk factors. Serum samples of adult healthy subjects (n = 200) were requested to Biobanco-iMM, Centro Académico de Medicina de Lisboa and patients with 5 stage CKD undergoing peritoneal dialysis (n = 97) were enrolled in a small crosssectional study. Serum calcium, phosphate, magnesium and echocardiogram analyses were performed for peritoneal dialysis patients. Serum tGRP levels were measured in both populations. We established the reference interval for tGRP as 531,1-3915,8 pg/mL in healthy adults, with the population under 42 years having higher tGRP levels than those over 42 (median: 1072,3 pg/mL and 702,2 pg/mL respectively; p < 0.01). In healthy participants, tGRP had a negative association with age subgroups (i.e., 18–30 years (young group), 31–46 years (middle aged group), and 47–67 years (eldest group)). In PD patients, tGRP serum levels were below the established reference intervals (median: 461pg/ml) and showed a negative association with calcium and phosphate (r= -0.791; r= -0.904; p< 0.01) and a positive association with magnesium (r= 0.405; p<0.01). Additionally, echocardiogram parameters for wall thickness and left ventricular mass index correlated negatively (r = -0.432; r = -0.384; p < 0.05) with tGRP serum levels in PD patients. This study demonstrates, for the first-time, age-dependent correlation of tGRP and establishes reference intervals of serum tGRP for age subgroups in a healthy population. In PD patients, tGRP correlates with cardiovascular markers and can identify future individuals at CVD risk.

Keywords: Gla Rich Protein; Vitamin K Dependent Protein; Chronic Kidney Disease; Peritoneal Dialysis; Cardiovascular Risk; Sandwich ELISA

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Molecular characterization of resistance mechanism of carbapenem-resistant *Escherichia coli* clinical strains[†]

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According to the World Health Organization, carbapenem-resistant gram-negative bacteria are of priority concern for their multidrug antimicrobial resistance profile. Furthermore, Escherichia coli, alongside Klebsiella pneumoniae, account for more than 90% of carbapenem-resistant Enterobacterales (CRE) and are widely spread worldwide. The European Centre for Disease Prevention and Control (ECDC) has also documented an increasing prevalence of CRE in Europe, with 43% of the countries reporting regional or inter-regional spread. However, data about carbapenemresistant E. coli epidemiology and resistance characterization is scarce. In this study, we aim to assess the resistance features of carbapenem-resistant E. coli in Portugal and compare them with the resistant traits reported in Europe and around the world. An original experimental study was performed with 40 carbapenem-resistant clinical strains of E. coli from healthcare-associated infections recovered from three hospitals in Portugal (August-2019 to June-2022). To characterize the extended-spectrum beta-lactamase (ESBL) genes and carbapenemase production they were characterized by Whole-Genome Sequencing (Illumina platform). As results, we found that 65.0% (n=26) had the KPC-3 class A serine carbapenemases genes and that the second most prevalent was the carbapenemase New Delhi metalo- β -lactamase NDM (n=10) with 25% of frequency, of which 90.0% were NDM-5 (n=9) and 10.0% were NDM-1 (n=1). OXA-48-type was the least prevalent gene, with one isolate (2.5%) OXA-48 producing and one isolate that was KPC-3, OXA-181 and extended-spectrum beta-lactamase (ESBL) CTX-M-15 co-producer. Moreover, we found only one isolate with no ESBL- or carbapenemase-producing genes. Finally, 32.5% (n=13) of the carbapenem-resistant isolates produced the ESBL CTX-M-15, mostly (54.0%) associated with the NDM gene. According to the available data at the literature, the most worldwide prevalent E. coli carbapenemase-producing are the NDM-1 and NDM-5, with numbers as high as 89% in Thailand. Second, we have either KPC (31% in Canada) or OXA-48-like genes (22,6% in Russia) depending on



the country. This data differs from Europe where the most prevalent carbapenemase gene is OXA-48-like with 43% of frequency, followed by NDM (20%) and KPC (14%). At lower rates, we also found VIM-1 genes in German. In Portugal, limited evidence is available. One previous study conducted in 2014 identified two carbapenem-resistant isolates and both had KPC genes. In conclusion, the present study has allowed to describe an unusual molecular resistant pattern with KPC being the prevalent gene found which should be address regarding the antimicrobial therapeutic choices and optimization. There is therefore an urgent need to provide recent data to characterize CRE in Europe, particularly in Portugal and addressing *E. coli*. Further studies should be performed in our country as well as in European soils to assess and better manage multidrug-resistant E. coli severe and difficult-to-treat infections.

Keywords: E. coli; Carbapenem-resistant; Enterobacterales; Antimicrobial stewardship; Antimicrobial resistance



Consumption of bioactive peptides from pork drycured ham in humans. Proteomic analysis of their biological activity on the cardiovascular system [†]

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The field of bioactive compounds, including biopeptides, has two distinct but interrelated main goals: 1) to understand the mechanisms of action at the physiological level and 2) to apply that knowledge to a clinical context. More specifically, most studies have analyzed the clinical effects in vivo, instead of investigating their mechanisms of molecular action. This is a major drawback in supporting the nutritional claims of new functional foods with bioactive peptides. Fifty-four volunteers with stage 1 prehypertension and/or hypercholesterolemia and/or basal glucose >100 mg/dL were recruited and randomized to pork dry-cured ham (n = 35) or cooked ham (placebo group; n = 19) for 28 days. The enrichment contains bioactive peptides characterized for their inhibitory activity of the angiotensin I-converting enzyme (ACE-I) and 3-hydroxy-3-metil-glutaril-CoA reductase (HMG-CoAR). In the current substudy, blood from 37 volunteers who consumed the intervention product (before and after) were assessed for proteomic analysis. Tryptic peptides were analyzed by capillarity mass spectrometry (MS/MS). The ion trap MS was operated in a data-dependent MS/MS mode where the five most abundant peptide molecular ions in every MS scan were sequentially selected for collision-induced dissociation with a normalized collision energy of 34%. All MS/MS samples were analyzed using Sequest (Thermo Fisher Scientific, San Jose, Ca) and X! Tandem. Scaffold (Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Samples were measured by different ELISAs Kits following manufacturer's instructions at different dilutions of the samples. The Metascape (https://metascape.org) is a reliable tool for functional analysis. 38 genes were loaded into the program, and terms with p < 0.01 were defined as significant. 272 proteins were identified after bioactive peptides enriched dry-cured jam consumption. Two different programs were used to identify the different proteins. ScaffoldQ program quantitative identify significantly different proteins expression. The results showed increased levels of ApoA-I (p<0.00010) and BTB/POZ domain-containing protein KCTD2 (p<0.05) after bioactive peptides consumption. On the other hand, there was a decreased of apoA-II levels (p=0.0062) after the consumption. Interestingly, by ELISA, ApoB/ApoA-I ratio was significantly decreased after biopeptides' consumption (p= 0.018). Moreover, MMP-8 levels were significantly decreased after the consumption (p=0.028). The resulting interactome identified different specifical genes grouped in different biological processes such as Ras activation upon Ca2+ influx trough NMDA receptor, peptidyl-threonine modification, regulation of actin filament-based process, RND3 GTPase cycle, regulation of actin filament-based process, RND3 GTPase cycle, regulation of lipid transport, protein phosphorylation, supramolecular fiber organization, and cell secretion. This study is the first to demonstrate the protective capacity of bioactive peptides of animal origin by modifying plasma proteomic profiles and their possible relationship with cardiovascular risk biomarkers. Through a new integrative strategy (proteomics, immunoassays and interactome), new mechanisms of action of bioactive peptides have been deciphered, which are fundamentally involved in cell signaling pathways. This will undoubtedly lead to a more extensive characterization of these possible proteins involved, with the aim of confirming the effect produced by the biopeptides but in an isolated system (in vitro).

Keywords: bioactive peptides, proteomics, immunoassays, interactome, molecular pathways, cardiovascular risk.



Consumption of last-line antibiotics during COVID-19 pandemic ⁺

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Coronavirus disease (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus. The pandemic raised concerns about the overuse of antibiotics in patients with COVID-19, that, despite being a viral disease, could increase the risk of antimicrobial resistance which is a major public health problem. However, there is a lack of data about this analysis. Therefore, we aimed to analyze the consumption of antibiotics for total care during the COVID-19 pandemic (2019-2023) in Portugal, with a special focus on last-line antibiotics such as carbapenems, due the impact in severe and difficult-to-treat bacterial infections. On April 21, 2023, we analyzed the data available on the (ESAC-Net), antimicrobial consumption dashboard available at https://www.ecdc.europa.eu/en/antimicrobial-consumption/surveillance-and-diseasedata/database. Moreover, the National Authority of Medicines and Health Products (INFARMED), a platform available at <u>www.infarmed.pt</u>, has also been analyzed. The European Center for Disease

Prevention and Control (ECDC) latest data states a global decrease (-13.6%) in the total antibiotic consumption in Europe between 2019 and 2021 (average of 20.84 to 18.00 DDD per 1000 inhabitants per day). A similar trend was observed in Portugal, with a reduction in total consumption from 19.29 to 15.28 DDD per 1000 inhabitants per day. However, according to INFARMED data, the carbapenems consumption in Portugal has increased significantly from March 2019 (18.29 DDD) to 2023 (21.83 DDD). In fact, the decrease in total antibiotic consumption during the year when the pandemic had a greater impact may have been a direct consequence of the lockdown, due to the many infection control measures that were put in place and the fact that people's contact with health services was greatly reduced. On the other hand, the increase in the consumption of carbapenems during this same period, can be explained by the unknowledge about the bacterial and viral co-infections for COVID-19 disease and that caused an over prescription of β -lactams broad-spectrum antibiotics as empirical treatment. It is now known that less than 5% of co-infections occurred. The main conclusion of our study is that the use of carbapenems, a last-line class of antibiotics, increased in Portugal during the early years of the COVID-19 pandemic. As a recommendation, additional antimicrobial stewardship and surveillance measures should be implemented immediately, mainly in hospitals, to reduce the impact of antimicrobial resistance.

Keywords: Last-line antibiotics; COVID-19; Consumption; Pandemic, Surveillance



An *in silico*-based drug discovery protocol to identify novel ERCC1-XPF complex inhibitors ⁺

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Alterations in key components of DNA repair pathways have been identified as prognostic markers and druggable targets in multiple types of cancers, including non-small cell lung cancer (NSCLC). Namely, overexpression of the ERCC1 involved in the Nucleotide Excision Repair (NER) pathway, has been linked with a poorer prognosis and response to chemotherapeutics. The primary goal of this work was to develop a strategy to identify small molecule inhibitors of the ERCC1-XPF complex capable of potentiating cisplatin efficacy in NSCLC therapy and overcome its resistance, using a structure-based virtual screening strategy (SBVS). The baseline of the SBVS platform involved a comprehensive structural and physicochemical characterization and in-depth analysis of the ERCC1-XPF complex, with a special focus on the XPF endonuclease for small-molecule inhibition. Crystal structures of ERCC1-XPF were selected to characterize the interface domain for their quality, the number of residues, region, and DNA binding. Each crystal structure was prepared using the MOEv09.2020 tool. Analysis of the ERCC1-XPF complex evidenced that the presence of Phe293 and its environment is essential for the stability of the complex. The Lys860 residue of XPF interacts with Phe293, being thus selected as the binding region for virtual screening calculations. Additionally, a library of small molecules with reported activity against the ERCC1-XPF complex was extracted from ChEMBL and prepared for screening in order to validate the molecular docking protocol. Molecular docking and virtual screening calculations were performed using the GOLD v2020.1 software (ChemPLP scoring function), which was then used to screen a large number of compounds from different databases (NCI, ChemBridge, and DrugBank). The results obtained were analyzed according to poses, binding affinities, and protein-ligand interaction fingerprints. The screening protocol incorporated the use of different filters (e.g., PAINS) to optimize the chances of identifying chemotypes with optimal drug-like properties. Previous insights obtained from the analysis of active compounds (e.g., molecular weight, hydrogen-bond donors, hydrogen-bond acceptors, rotatable bonds, and logP) were determinant for compound selection, and 105 new putative inhibitor molecules were then chosen and acquired for further studies. Our findings illustrate the importance of smallmolecule key features in ERCCI-XPF inhibitory activity and provided important insight that allowed the identification of potential XPF pharmacological inhibitors with novel chemotypes.

Keywords: ERCC1-XPF; DNA repair; molecular docking; structural analysis; Virtual Screening



Factors conditioning the stage of oral carcinoma at diagnosis ⁺

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Socioeconomic factors play an important role in access to healthcare, particularly oral health. They can therefore have a major impact on quality of life. Our primary objective was to assess the relevance of these factors, among others, in relation to the stage of oral cancer at the time of diagnosis. Secondary aims were to relate the pathological stage at diagnosis to the time between the onset of symptoms and the first appointment and the start of treatment, to see how this affects prognosis. The study also aimed to determine which professionals referred patients to the study centre, the Francisco Gentil Portuguese Institute of Oncology in Lisbon (IPOLFG) and evaluate the importance of oral carcinoma early diagnosis. From the analysis of the clinical files between 2018 and 2023 and the questionary forms applied during medical appointments, it was possible to compile data such as sex, age, residence area, and some predisposing factors for the pathology - smoking, alcohol consumption, HPV and other infectious diseases, poor oral hygiene, and previous history of oral neoplasm. The date of diagnosis and its stage was evaluated according to the TNM classification (Tumor, Node, Metastasis), and the undergone treatments were noted. Briefly, the results showed that the sample of the population studied with oral cancer symptoms in IPOLFG (N=75) was constituted essentially by patients older than 60 years old (69.3%), the majority males (66,7%), and with low income (54.6% less than 10.000 €/year). Most patients were smokers (61%), while some were heavy smokers (43%) consuming more than 20 cigarettes/day. About 61% had alcoholic habits, and 51% were heavy drinkers. These patients with exaggerated alcohol habits were all male. Overall, 55% were not undergoing any rehabilitation treatment and 42% were undergoing rehabilitation treatment. The patients without alcoholic habits (39%) were mostly female. Oral cavity neoplasms often result from repetitive trauma, such as ill-fitting dentures. In the overall sample, 44% had previous conditions, of which 9% had infectious diseases like HPV, HIV, or hepatitis. 10% of the sample had poor eating habits (as they were obese), and poor nutrition is considered a risk factor for carcinoma of the oral cavity. A small percentage had a family history of cancer or of premalignant lesions (5%), or of oral lesions with malignant potential (7%), such as leukoplakia. Regarding the referral to the IPOLFG, 64% were made by Dentists, 25% were attributed to General Practitioners and 9% by other specialties. The study led to the confirmation that socioeconomic factors and social behavior have a high impact on oral health and the risk of oral carcinoma in its various forms. The predominating risk factors are smoking habits and alcohol consumption, and their synergetic effect may increase the overall cancer risk in patients. In short, most injuries are discovered by the Dentist, since he is the one who most carefully inspects the oral cavity and can often diagnose the neoplasia early, resulting in less invasive treatments.

Keywords: oral cancer, risk factors, socioeconomic factors and diagnose

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Oral Health in psychotropic medicated outpatients of the Lisbon Psychiatric Hospital Centre (CHPL) ⁺

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In Portugal, 22.9% of the total population have an experience a mental health disorder at some stage in their life. There is evidence that people with a serious mental illness experience worse oral health outcomes than the general population. People who have experienced a mental health disorder are a heterogeneous group commonly exhibit many factors which may contribute to poor oral health: xerostomia caused by psychiatric medication, lack of motivation for self-care, poor oral hygiene and difficulties to access dental care. The aim of this study is to assess the prevalence of dental caries and self-care behaviour in psychotropic medicated outpatients of the Lisbon Psychiatric Hospital Centre (CHPL). A cross-sectional study was conducted for a period of 4 months (March-June 2022) at the outpatients department of the General Psychiatry at the CHPL. The inclusion criteria for the patients were as follows: had the psychiatric diagnosis according the International Classification of Diseases-10; have had the psychiatric condition for at least 1 year; have taken antipsychotic medication for at least 1 year; being treated as an outpatient; and over 18 years old. A sample of 60 outpatients selected randomly from the psychiatry department of CHPL of both genders, aged between 27 and 72 years who consented to participate in this study. The prevalence of dental caries was assessed by decayed, missing and filled teeth index (DMFT). Subsequently, a questionnaire was applied regarding sociodemographic variables, psychiatric pathology, and oral hygiene habits. Data were submitted to descriptive analysis using IBM SPSS Statistics® v.28 software. The prevalence of dental caries was 98, 3% and DMFT score was 18, 03 ± 9 , 39 among our population. Overall DMFT scores ranged from 0 to 32, whereas the mean of decayed (D) score is 3,63±3,9, the mean of missing (M) score is $12,81\pm11,03$ and the mean of filled(F) score is $1,56\pm2,95$. According the tooth brushing frequency, 40, 0% of the population brushed the teeth twice daily. Among 63,30% of population had the last dental visit over a year. DMFT index is classified in five levels: 0.0-4.9 very low, 5.0-8.9 low, 9.0–13.9 moderate, 14.0–17.9 high, and more than 18 very high. Some study determined the dental caries was prevalent in 87,3% of psychiatric outpatients in India, the mean of DMFT scores is 4,06 and the mean of decayed (D) score is 3,4 and other study conducted in Haryana in a population of schizophrenic patients demonstrated that among 72% and 3% of the subjects brushed their teeth once and twice daily. Despite the prevalence of suboptimal oral health among mental health disorder patients compared with the general population, the dental services are often underutilized by this heterogeneous group due the stigma, helplessness, low self-esteem and low -income. The disparity of poor oral health are likely to be multifactorial and may include psychotic medication, personal capability, systemic issues and further research is needed to understand the barriers of low rates of oral heath self-care in this heterogeneous group.

Keywords: Psychotic disorders, Oral health, Dental caries

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Body mass index as independent predictor of diaphragmatic function in amyotrophic lateral sclerosis [†]

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive disease that causes respiratory dysfunction, mainly due to the diaphragm weakness, the most important muscle sustaining ventilation. The phrenic nerve motor response amplitude is used to estimate the number of the motor units in the diaphragm. Previous studies proved that phrenic nerve motor responses are correlated with Force Vital Capacity (FVC). The interaction between ALS and metabolism has been studied, and metabolic abnormalities have been found. Patients tend to lose weight, but the pathophysiological mechanisms underlying this are not fully understood. Weight loss and decreased respiratory function are poor prognostic indicators in ALS, but their relationship is unknown. The current study sought to determine the relationship between the decline of diaphragmatic function and the decline of Body Mass Index (BMI) in ALS patients throughout the disease. Therefore, the following inclusion criteria were used to select retrospective data from ALS patients followed in our unit: onset age above 18 years, and functional evaluations using the revised ALS functional rating scale (ALSFRS-R), BMI, and phrenic amplitude assessed at least three times (T1, T2, T3). The decline of the parameters determined between T1-T2, T2-T3, and T1-T3 was used in a longitudinal analysis. Non-parametric correlations and multiple linear regression models were run with phrenic amplitude decline as the dependent variable, and BMI at first visit, BMI decline, ALSFRS-R decline, age at onset, disease duration at T1, diagnostic delay, and phenotype as independent variables. The 72 ALS patients (51 males, 60 spinal-onset) included had a mean age at onset of 59±8 years, a mean BMI at 1st visit of 24.9 ± 2.3 kg/m2, a mean disease duration at T1 of 15±8 months and a mean ALSFRS-R at T1 of 44±2. The mean interval between T1 and T2 was 7 months, between T2 and T3 was 7 months, and between T1 and T3 was 14 months. Phrenic amplitude decline was strongly correlated with BMI variation between T1-T3 (correlation coefficient=0.396, p=<0.001), but there was no significant correlation between T1-T2 and T2-T3. BMI variation (p=0.004) and ALSFRS-R total score variation (p=0.060) was identified as independent predictors of phrenic amplitude decay between T1 and T3. Consequently, our findings show that BMI decline over a longer interval of time is a predictor of diaphragmatic function loss in our ALS population.

Keywords: Amyotrophic lateral sclerosis, diaphragmatic function, phrenic nerve, body mass index



Blood Neutrophil-to-Lymphocyte ratio predicts Survival in Amyotrophic Lateral Sclerosis patients⁺

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative, progressive, and fatal disease, characterized by the death of motor neurons. Consequently, these patients suffer from muscle weakness and atrophy, spasticity, dysarthria and sialorrhea, functional impairment, and in more advanced cases, respiratory failure. Neuronal death results from several pathogenic mechanisms, with inflammation being one of the processes related to the neurodegeneration described in ALS. It has also been reported a larger number of inflammatory cells in the tissue of ALS patients. Therefore, since the inflammatory process is potentially associated with prognosis, it is pertinent to identify biomarkers that are easily accessible. The blood count is a routine test from which a considerable number of parameters altered by the presence of an inflammatory process can be extracted. With the use of this blood sampling, it is possible to determine the neutrophile-to-lymphocyte ratio (NLR), which reflects a dynamic relationship between innate (neutrophils) and adaptative cellular immune response (lymphocytes) during illness and various pathological stages. NLR is described as a reliable and economic marker, as well as a robust prognostic biomarker for predicting patient survival outcomes in many diseases, such as cancer, major cardiac events, ischemic strokes, infections, pulmonary disease, and autoimmunological inflammatory disorders. In the specific case of ALS, this parameter may have implications on the implementation of appropriate measures, such as ventilatory support, consequently improving the clinical care provided and facilitating the stratification of patients in clinical trials. Therefore, the purpose of this study was to observe and evaluate Neutrophile-to-Lymphocyte ratio in ALS as a predictor of survival in ALS patients. In this retrospective study we aimed to analyze data from 274 patients followed in our center. Neutrophileto-Lymphocyte parameters were quantified from a standard complete blood count, and the NLR was calculated as the ratio of the neutrophile count to the lymphocyte count. Patients were clinically evaluated at the time of blood sampling and longitudinally thereafter, applying the revised ALS functional rating scale. Standard respiratory function tests were also performed in these patients, to evaluate the functional but also respiratory progression of the disease. NLR was then explored as an indicator of survival applying Cox regressions (p < 0.05). The sample of ALS patients consisted of Our results suggested that high NLR levels were associated with a lower survival rate (p = 0.043). However, by multiple regression NLR had no statistical significant correlation with ALS rate of decline and respiratory decline. Although this negative correlation significantly shows the potential predictor NLR could be in ALS survival, it is important to evaluate these parameters in different time points and disease stages making it possible to better understand the functions behind the immune response in pathomechanisms of ALS. We have found promising results suggesting that NLR could represent a reliable predictor of ALS survival in our population.

Keywords: Amyotrophic Lateral Sclerosis, Inflammation, Biomarkers, Blood Biomarkers, Inflammatory Biomarkers, Neutrophile-to-Lymphocyte ratio



P.75

Search for bioactive compounds with antioxidant activity in seaweed species from the Portuguese coast [†]

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Research on bioactive compounds in seaweeds has been the subject of several research studies in recent years. The Portuguese coast has a huge diversity of seaweed species, which offer great potential for studying bioactive compounds. Antioxidant molecules present an important role in cell health by protecting against oxidative damage, which can induce cell death. Seaweeds are known to contain some molecules with antioxidant properties, but these are often left unidentified or only classified in terms of its class. Thus, the aim of this work is to obtain several extracts from seaweeds collected in the Portuguese coastline, containing compounds with potential antioxidant activity. Methods: the species selected to perform this work were: Palmaria palmata (L.) F. Weber & D. Mohr, 1805; Chondrus crispus Stackhouse, 1797; Codium tomentosum Stackhouse, 1797; Osmundea pinnatifida (Hudson) Stackhouse, 1809; Himanthalia elongata (Linnaeus) S.F. Gray, 1821; Bifurcaria bifurcata R.Ross, 1958; Fucus spiralis Linnaeus, 1753; Porphyra umbilicalis Kützing, 1843; Ulva intestinalis Linnaeus, 1753; Cystoseira tamariscifolia (Hudson) Papenfuss, 1950; and Asparagopsis armata Harvey, 1855. Different extraction methods were tested after freeze-drying the seaweed species and grinding it to a fine powder fraction. Extractions were also performed on fresh seaweed samples. Some of the extraction methods used were: Bligh-Dyer method (126 to 26 g of algae/ml solvent mixture), two-phase extractions using ethyl acetate:methanol (1:1 v/v; 40 g/L), destilled water:methanol (1:1 v/v; 40 g/L), and Phosphate buffered saline (PBS):methanol (1:1 v/v; 40 g/L), all assisted by ultrasound. Supercritical CO_2 extraction was also carried out on seaweed samples and tested. The total protein, lipids and phenolic compounds were analyzed in the samples obtained from the various extraction methods. The antioxidant activity was assessed using the Ferric Reducing Antioxidant Power (FRAP) assay, the total protein followed the Bradford method, the total lipid content followed the gravimetric method, and the total phenolic content was determined quantitatively using the Folin Ciocalteu reagent, with gallic acid as the standard. Results: The methanolic extracts of C. tamariscifolia presented the highest antioxidant activity $(1.168 \pm 0.05 \text{ mg})$ TE/g dry weight), followed by *P. umbilicalis* (0.279 \pm 0.001 mg TE/g dry weight). The methanolic extracts of O. pinnatifida showed the highest content of total protein $(4.0 \pm 0.18 \text{ mg soluble protein/g})$ dry weight) compared to the other species, while the methanolic extracts of *P. palmata* showed the highest phenolic content (76.88 \pm 0.001 µg GAE/g dry weight). The highest amount of total lipids was found in *P. umbilicalis* (0.4 ± 0.002 g/g dry weight). Further work will characterize the most promising extracts using different techniques (e.g., LC-MS/MS, GC/MS) to identify molecules which can be responsible for the antioxidant activity.

Keywords: seaweed bioprospection; biomolecules; marine natural products; bioactive compounds; antibacterial activity; blue biotechnology

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Electrostatic assembly of Metalloprotein to magnetically oriented Pf1 virus alignment matrix⁺

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This work aims to develop and study novel magnetically induced and self-assembling biological materials. The application of a strong external magnetic field can create a macroscopic alignment matrix that, upon electrostatic binding, spatially organises an ensemble of protein molecules (Figure 1). We have developed a partially aligned material based on the electrostatic assembly of the Pf1 virus and a metalloprotein (cytochrome c). The Pf1 virus can be magnetically aligned (> 7 Tesla) to produce the aligned matrix. This bacteriophage has a tubular structure ($I=2 \otimes m$, $\emptyset=6.7$ nm) with a single strain DNA inside and a capsid of 7620 subunits of 46 amino acids (one subunit per DNA base). The virus surface is strongly negatively charged (-0.475e/nm2, pI=4.0) due to the presence of 3 aspartic acids and no other charged residues per subunit. Cytochrome c (horse heart) has a positive surface charge (pI = 10.2). Experimentally, we have determined a reversible binding between Pf1 bacteriophage and cytochrome c at ~120mM ammonium acetate pH 6.8 at 25°C with a specific stoichiometry that produces a neutrally charged complex. This organised complex was characterised by AFM, DLS, zeta potential, diffusion-ordered NMR and thermodynamic statistical Monte Carlo simulations, revealing the details of the salt dependent Pf1|cit c association and its mechanism. This new method will provide the relationship between structural and spectroscopic data to be established for more complex metalloprotein molecules. This new experimental methodology will allow the creation of molecularly aligned macroscopic samples that can be observed by an array of spectroscopic techniques revealing unprecedented characterisation of enzymes energy landscape.



Figure 1. Experimental protocol diagram of matrix alignment Pf1 virus magnetic on strong magnetic field (>7 Tesla) and electrostatic assembly metaloprotein cytochrome upon dialysis (I <0,5 M).

Keywords: Pf1 bacteriophage; cytochrome c; orientation selection

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Assessment of the nutritional status of 2-5 years old children in Almada: a cross-sectional study ⁺

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The first years of life are critical for establishing healthy dietary habits, appropriate neurocognitive development, and developing protective factors against noncommunicable diseases. Growth monitoring becomes essential for confirming the child's healthy development or identifying a potential nutritional or health concern early on. Because weight and height are regarded major indices of growth in children and adolescents, growth curves are an essential tool for analyzing and monitoring child growth. The CDC growth charts depict the gender-specific BMI-per-age percentile curves and can be used to track the growth of children and adolescents aged 2 to 19 years. The primary goal of this study was to characterize the nutritional status of Almada children aged 2 to 5 years old using weight, height, and BMI measurements. A descriptive cross-sectional study was carried out in Almada, Portugal, in February 2023. The children in the study ranged in age from 2 to 5 years. Anthropometric measurements, such as weight and height, were taken with verified standard instruments. Weight for stature percentiles for children aged 2 to 5 years were generated using a children growth chart calculator from the 2000 CDC recommended growth charts (weight for stature - kid 2 to 20 years). The percentile was determined separately for boys and girls. According to the CDC, the 5th percentile represents underweight, the 5th - 85th percentile represents a healthy weight, and the 85th percentile represents overweight/obesity. The study involved 100 children, comprising 49 children aged 24-36 months (22 boys, 27 girls) and 51 children aged 3-5 years (20 boys, 31 girls). Globally, 75% of the samples from both the 24-36 month and the 3-5 year age groups were healthy weight, 21% were overweight/obese, and 4% were underweight. In the 24-36 months group, girls had 70.37% (n = 19) of healthy weight, 29.63% (n = 8) of overweight/obesity; boys had 4.54% (n = 1) of underweight, 81.82% (n = 18) of healthy weight, and 13.64% (n = 3) of overweight/obesity. The girls mean weight was 14.27 ± 1.94 kg and the boys mean weight was 14.59 \pm 1.37 kg in the 24-36 months group. In the 3-5 year age group, girls had 3.23% (n = 1) of underweight children, 74.19% (n = 23) of healthy weight children, and 22.58% (n = 7) of overweight/obesity children; boys had 10% (n = 2) of underweight children, 75% (n = 15) of healthy weight children, and 15% (n = 3) of overweight/obesity children. The girls mean weight was 17.75 \pm 2.06 kg and the boys mean weight was 16.87 \pm 1.80 kg in the 2-5 years group. In conclusion, the current study found that the majority of children aged 2 to 5 were of a healthy weight. Despite this, the nutritional assessment revealed that 28% of the children were overweight or obese. To ensure a healthy body weight, it is still necessary to check children's nutritional status. More school-based nutrition intervention should be implemented to promote healthy eating habits and nutritional status.

Keywords: Children; BMI; Weight for stature; nutritional status; nutrition



Epilepsy patients and recurrent admissions to the emergency department: causes and means for reduction ⁺

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Epilepsy is a chronic neurological disease that affects approximately 50 million people of all ages globally and 2.6 to 6 million people in Europe. People affected by this disease have a risk of premature death three times higher than that of the general population, despite being widely known and studied. Data on the prevalence of epilepsy in Portugal are scarce and outdated, which highlights the need for recent and high-quality data to describe the Portuguese situation regarding epilepsy. Patients with a previous diagnosis of epilepsy often visit the emergency department (ED) instead of a doctor. This inappropriate use of emergency services is estimated to range from 20% to 40% worldwide and is the cause of several problems, including increased costs and reduced continuity of care, leading to the overuse and exhaustion of health services. This study aimed to analyze ED use in patients with epilepsy and to determine the prevalence of patients who could be redirected to other services. The project consists in one-year retrospective cross-sectional study to analyze the causes of hospital readmissions in patients with diagnosed epilepsy. There is a direct partnership with two out of ten public hospital institutions in the Lisbon Metropolitan Area that provide care to patients with epilepsy (Hospital Garcia de Orta and Hospital Egas Moniz), with access to patients and clinical records. This project includes a qualitative analysis through a questionnaire distributed in the participating hospitals, which aims to learn the most frequent conditions leading an epilepsy patient to seek the ED. The analysis of data from patient records will allow confirmation of the information provided by the questionnaire, decreasing the bias associated with retrospective questionnaires. The answers provided by both tools will allow us to identify the main reasons for ED use and define strategies to decrease ED overcrowding by taking patient opinions into account. Considering the prevalence data of epilepsy in Portugal (0.24% in 2016) and the population with epilepsy in the Lisbon Metropolitan Area, we calculated the population that each hospital institution could receive, resulting in a total of 681 patients. Thus, with a 95% confidence interval, our sample consists of 246 patients with epilepsy per hospital. With this protocol, we hope to reduce patient mortality, dissatisfaction, poor outcomes, readmissions, and delayed assessment. This project aims to optimize adherence to best practice guidelines and reduce workload.

Keywords: epilepsy; emergency department; retrospective study.

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Validity and reliability of the "Australian pelvic floor questionnaire" for the Portuguese population[†]

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Pelvic floor dysfunctions have a negative impact on women's quality of life. These dysfunctions include mostly: urinary incontinence, fecal incontinence, and pelvic organ prolapses. The Australian Pelvic Floor Questionnaire is a self-administered measurement instrument used to assess pelvic floor function and contains four domains: Bladder Function, Bowel Function, Pelvic Organ Prolapses, and Sexual Function. It assesses frequency, severity, and impact of pelvic floor symptoms on quality of life. The aim of this study was to translate, cultural adapt and validate the original version of the Australian pelvic floor questionnaire (APFQ) into European Portuguese. This is a methodological, nonexperimental study. The process of cultural and linguistic adaptation and validation was carried out in three stages: semantic equivalence, content validity and psychometric properties evaluation. The first stage was translation and back-translation of the instrument. Content validity was assessed by an expert panel of Women's and pelvic Health expert Physiotherapists. For pre-test a sample of 9 women with pelvic floor dysfunction participated in cognitive assessment of the questionnaire. Last stage consisted of psychometric properties evaluation. A sample of 50 women with pelvic floor dysfunction completed the questionnaire on two occasions, with a seven-day apart interval. Reliability (internal consistency, reproducibility, and standard error of measurement), validity (of construct) and ceiling effects assessment was evaluated. In the first phase of the process, semantic and content equivalence was obtained. The pre-test sample considered the questionnaire clear and suitable for the population with pelvic floor dysfunction (PFD). APFQ psychometric properties, showed a high Cronbach's alpha, for bladder function domains 0.837, bowel function 0.756, pelvic organ prolapses 0.840 and sexual function domains of 0.756 and a total score of 0.714. In reproducibility, ICC domains values ranged from 0.934 to 0.976 with Total Score of 0.948. The APFQ was culturally, linguistically adapted and validated for European Portuguese. This measurement instrument contributes to the systematic integration and execution of clinical practice, enabling a more objective assessment of information gathering for decision-making by both professionals and patients themselves. The APFQ serves as an effective tool for collecting information on a topic that many women feel embarrassed to discuss or that, in some cases, remains a taboo subject. Furthermore, this research facilitates result comparisons with other populations, thereby enhancing the quality and advancement of scientific investigation and improving healthcare provision. The Portuguese version showed acceptable values of validity and good reliability. APFQ can be used both in clinical evaluation and in research of pelvic floor dysfunctions.

Keywords: Pelvic Floor Dysfunction, Australian Pelvic Floor Questionnaire, European Portuguese, Woman



Metal removable parcial dentures, a survey in Egas Moniz Dental Clinic ⁺

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According to the OMD's Oral Health Barometer 2022, there is a slight improvement but at least 70% of the Portuguese population lacks natural teeth and 38.3% use removable dentures (RD). Metal removable partial dentures (RPDs) remain a common long term prosthodontic treatment in Portugal, while acrylic removable partial dentures are considered a provisional treatment. Ztmannn et al, in a systematic review on the type of dental rehabilitation performed in Europeans, concluded that although fixed restorations are more frequent in younger age groups, RD are the dominant type of treatment in older groups. The aim of the present study was to evaluate the prevalence of treatment with RPDs in a sample of patients of the Egas Moniz Dental Clinic, according to Kennedy classification, age and sex. The present study was submitted and approved by the Clinical Directorate, the Scientific Committee and the Ethics Committee, approval document 424. All clinical charts (240) of the prosthodontic clinic between September 2014 and June 2015 were evaluated and characterized by: age, sex, Kennedy classification and type of rehabilitation completed. Descriptive statistical analysis with crosstabs procedures was used to check frequencies, using SPSS Statistics 22 software. A total of 48 metal frame prostheses were delivered. Patients ranged in age from 30 to 88 years, with a mean age of 59 years. Females were predominant (68.4%) and younger. Kennedy Class III rehabilitation was most common in the maxilla (46.2%) and mandible (53.8%), while there was no Class IV (0%). There was a higher prevalence of RPDs in the mandibular arch (55.9%) in women. There was no difference in prevalence in men. The number of teeth replaced was higher in women (5.06) than in men (3.86). The mean age was 59 years. Of these, 68% were women. Similar results were found by Carneiro et al in Portugal, Souza et al in Brazil and Pun et al in Greece. The mean number of replaced teeth was higher in women. The predominant edentulous class was Kennedy class III and the lowest class IV, similar to the findings of Carneiro Souza and Pun. Most of the edentulous arches were located in the mandible, as reported by Naveed et al, Patel et al, Pellizzer et al, Polychronakis et al. Dental schools have a key role in developing skills of future professionals in collecting clinical data and rehabilitation planning. The collection of the data would be of profound clinical interest for further scope of improvement in the teaching and planning of oral rehabilitation.

Keywords: Kennedy classification, partial edentulism, tooth loss, RPD, metal framework.



Assessment of xerostomia in outpatients of the Lisbon Psychiatric Hospital Centre (CHPL)⁺

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Saliva is a biochemically complex fluid containing a unique mixture of water, proteins, alycoproteins, and ions that aid several functions, including lubrication of oral tissues, dental remineralization and antimicrobial activity. The saliva acts as a physical barrier because of its numerous immune and nonimmune defense components. Salivary secretion dysfunction, mostly caused by adverse drugs effect may cause mouth dryness (xerostomia) because of hyposialia. Antipsychotics and other medications, including first-generation antipsychotics, second-generation antipsychotics, and anticholinergics may often disturb saliva section and cause drug induced hyposialia. The aim of this study is to assess the prevalence of hyposalivation and xerostomia in psychotropic medicated outpatients of the Lisbon Psychiatric Hospital Centre (CHPL). A crosssectional study was conducted for a period of 4 months (March-June 2022) at the outpatients department of the General Psychiatry at the CHPL. The inclusion criteria for the patients were:(1) had the psychiatric diagnosis according to the International Classification of Diseases-10;(2) have had the psychiatric condition for at least 1 year; (3) have taken antipsychotic medication for at least 1 year; (4) being treated as an outpatient; (5) over 18 years old. A sample of 60 outpatients selected randomly of both genders, aged between 27-72 years who consented to participate in this study. Approved by the Ethics Committee of the Lisbon Psychiatric Hospital Centre and approved by the Egas Moniz Ethics Committee with the approval number 1126. Subsequently a questionnaire was applied regarding sociodemographic variables and SXI-PL. Sialometry was performed for unstimulated (USFR) and stimulated (SSFR) salivary flow rates. Hyposalivation was considered when USFR<0.1 mL/min and/or SSFR<0.7 mL/min. Data were submitted to descriptive analysis using IBM SPSS Statistics® v.28 software. The prevalence of hyposalivation was 16.7% and xerostomia was 23.3% among our population. Overall SXI-PL scores ranged from 5 to 15 with a mean of (7.41 ± 2.28) and the symptom that showed higher severity of xerostomia was "My mouth feels dry" (1.78 ± 0.81) . The mean scores of SXI-PL was higher in patients with normal saliva flow (7.60±2.26). The results demonstrate no statistically significant correlation between SXI-PL scores and hyposalivation (p>0.05. The SXI-PL score was higher in normal saliva flow condition among our population. Among patients with mental disorders, often have behavioral patterns impairment, an inability to distinguish symptoms of a concurrent physical illness and do not perceive the need and perception of their oral status. Some studies demonstrated the evidence presented for oral reactions being drug-induced is variable, some hospitalized patients who experienced dry mouth symptoms have been shown to be taking more than two kind of drugs such as cardiovascular, psychiatric and allergy drugs than those who did not complain of dry mouth. Some studies referred the most severe symptom of xerostomia reported was "My mouth feels dry". The disparity of poor oral health of people with common psychological disorders remains a largely forgotten problem and worrisome such as the iatrogenic effects of antipsychotic medication on oral health. Greater awareness of these effects could help to protect against the poor outcomes seen in chronic psychosis and relief the burden of the implications for public health.

Keywords: Psychotic disorders, Hyposalivation, Xerostomia

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Risk factors associated with mini-implant failure: a retrospective study $^{\rm t}$

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This study aimed to evaluate the failure rate of mini-implants used at the orthodontic external clinic, Egas Moniz School of Health and Science. A retrospective cross-sectional investigation including 232 TADs inserted in 125 consecutive patients always with an immediate loading protocol. The examined variables were; gender, age, Angle' classification, presence of pathologies, medication, smoking, receiving jaw, placement side and insertion site. Descriptive statistics and inferential analysis were performed revealed that a six-month failure rate of the used TADs was 25%. A significant association was found between being a smoker and the failure rate (p=0.036), and the placement site (where the interradicular region had the highest success rate) (p=0.003). In contrast, there was no significant association (P>0.05) between the following variables and the level of MI failure: gender, presence or absence of pathologies, medicated or non-medicated, type of malocclusion, recipient jaw, and direction of movement aided by the MI.

Keywords: miniscrew, orthodontic, mini implant, skeletal anchorage, temporary anchorage device



Are Food Producing animals a source of multidrug-resistant *E. coli* and *Salmonella* spp.?⁺

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Zoonoses have been responsible for several human diseases, many associated with the consumption of contaminated food, where pathogenic and drug-resistant Escherichia coli and Salmonella spp. are among the principal bacterial agents. To understand the epidemiology and population structure of these zoonotic agents in Portugal, we aimed to assess the role of foodproducing animals as potential transmission vehicles of these bacteria to Humans. During 6 months, fecal samples were collected in different Portuguese slaughterhouses. After bacterial isolation in nonand selective media, serotyping of Salmonella isolates and E, coli virulence factors detection were performed. Anti-microbial susceptibility was tested for a sub-set, by the disk diffusion method and interpreted according to EUCAST guidelines. A total of 252 samples (123 from pigs, 82 from chickens and 47 from turkeys) were studied, mostly from Centro and Lisboa e Vale do Tejo Regions. Salmonella spp. (S. enterica enterica serovar Cremieu) was only detected in one turkey sample, while E. coli was identified in all studied samples from the three ani-mal species, with Shiga toxinproducing E. coli (STEC; stx2e gene) detected in two pig samples. Preliminary antimicrobial resistance results to 18 antibiotics showed that 75 out of 101 pig E. coli isolates were resistant to at least one tested antibiotic (mostly to tetracycline), where 38 of them displayed a multidrugresistance (MDR) profile, 42.1% to three (e.g., beta-lactams, cephalosporins, macrolides), 44.7% to four (e.g., beta-lactams, cephalosporins, folate inhibitors, sulfonamides), and 13.2% to five or more antibiotic classes (e.g., amphenicols, aminoglycosides, beta-lactams, fluoroquinolones, folate inhibitors, sulfonamides, tetracyclines), including to some critically important antimicrobials to humans (like azithromycin, cefepime or ciprofloxacin). Interestingly, one of these MDR iso-lates was a STEC. So far, testing on a small number of isolates recovered from chickens and turkeys also revealed multidrug-resistance, foreseeing a high prevalence as already observed in pig isolates. Overall, our preliminary findings revealed the presence of MDR E. coli isolates in fecal samples of pigs, chickens and turkeys slaughtered for human consumption. This is an alarming scenario as the resistance genes responsible for these phenotypes can be transmitted between bacteria, but also to other animals and humans.

Keywords: Zoonotic Bacteria; Multidrug-resistance; Escherichia coli; Salmonella; Food-producing animals, One Health.



Cyclodextrin-containing contact lenses loaded with triamcinolone acetonide to treat diabetic macular oedema [†]

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The prevalence of diabetic eye disease is continuously rising worldwide and encompasses a variety of ocular conditions such as diabetic retinopathy, diabetic macular oedema (DMO), and glaucoma. Triamcinolone acetonide (TA) is a corticosteroid drug that can be employed in the management of DMO and is administered intravitreally. Despite being the most efficient route to deliver the drug to the posterior part of the eye, it may induce severe ocular complications. Contact lenses (CLs) constitute an advantageous platform for the topical release of ophthalmic drugs by providing prolonged levels in tear fluid. However, the lack of interaction between CLs and lipophilic steroids hampers its ability to load therapeutic amounts. Herein, we incorporate hydroxypropyl- β cyclodextrins (HP- β -CDs), cyclic oligosaccharides with lipophilic cavities, to improve drug loading and release from CLs. Two approaches were evaluated: the addition of HP- β -CDs to the monomer's solution before polymerisation (i-CD) and a post-treatment of the CLs with a HP- β -CD solution (p-CD). The effect of the HP- β -CDs on the hydrogel, as well as of the sterilisation by high hydrostatic pressure (HHP) on the material's properties, namely in solvent uptake, transmittance, and stiffness, was studied. TA was loaded by soaking in a supersaturated drug solution (0.3 mg/mL). The drugloaded hydrogels were used for in vitro drug release tests in sink conditions. Adsorption of two of the main proteins of lacrimal fluid (albumin and lysozyme) onto the materials was studied and biocompatibility was evaluated through cytotoxicity and irritability assays. The permeability of TA through cornea and sclera was assessed ex vivo. Finally, the anti-inflammatory activity of TA-eluting CLs was investigated. The designed hydrogels revealed physical properties suitable for CLs. Functionalisation with HP- β -CDs endowed the hydrogels with a stronger affinity for TA and sustained release for one day. HHP sterilisation promoted the formation of cyclodextrin-TA complexes within the CLs, improving the drug loading capacity of the hydrogels. Indeed, HHP sterilised i-CD CLs doubled the affinity of TA to the network and resulted in the highest amount loaded (10 mg/g dry lens). Cytotoxicity in human corneal epithelial cell cultures and HET-CAM irritability tests confirmed the safety of the therapeutic CLs. TA released from the CLs decreased the secretion of proinflammatory cytokines (IL-6 and TNF-alpha) by LPS-stimulated macrophages. Overall, the CLs revealed to be suitable candidates for the topical ocular application of TA as an alternative delivery system to intraocular injections.

Keywords: keyword 1; triamcinolone acetonide; HP-β-cyclodextrin; drug-eluting contact lens; diabetic macular oedema; high hydrostatic pressure sterilisation

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Emotional Intelligence and Life Satisfaction in a Sample of Adult Victims and Non-Victims of Violence [†]

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Emotional intelligence is understanding, controlling, and regulating emotions in oneself and others, influencing the perception of quality of life. Individuals who suffered victimization experiences show themselves more vulnerable and with lower emotional intelligence, increasing stress and dissatisfaction with life. The main objective of this study is to: identify the relationship between emotional intelligence and life satisfaction; compare a sample of victims and non-victims of violence in adulthood and a sample of men and women regarding emotional intelligence and life satisfaction; analyze the variables that explain the variance of satisfaction with life in a sample of the Portuguese population. This study comprised a sample of 144 individuals, aged between 18 and 77 (M=36.97, SD=15.87). Nearly half of the sample (n=66) reported being victims of violence in adulthood. The study design is cross-sectional with a non-probabilistic sample. The study was disseminated through personal and social contact networks. Participants answered online the sociodemographic questionnaire, the Wong and Law Emotional Intelligence Scale (WLEIS: Self-emotion appraisal, Others' emotion appraisal, Use of emotion, and Regulation of emotion), and the Satisfaction with Life Scale (SLWS). All participants were granted their consent to participate in this study. The study followed the ethical principles outlined in the Declaration of Helsinki and was approved by the Institutional Review Board. The results show statistically significant positive correlations between satisfaction with life and emotional intelligence (r=.541, p<.001), self-emotion appraisal (r=.428, p<.001), others' emotion appraisal (r=.363, p<.001), regulation of emotion (r=.364, p<.001), and use of emotion (r=.511, p<.001). When emotional intelligence increases, life satisfaction also increases. Those outcomes align with other studies, which showed that individuals with higher emotional intelligence are more satisfied with their lives and are more able to understand and pay attention to their emotions, proving their greater personal fulfillment and achievement. The results heightened significant differences between victims and non-victims of violence in adulthood regarding satisfaction with life and emotional intelligence. In other studies, victims also reported lower life satisfaction. High emotional intelligence makes individuals more motivated, solve problems and achieve goals using emotions. Thus, victims with higher emotional intelligence may be able to get through abusive situations and feel less anxiety and fear. Furthermore, differences were found between men and women in evaluating other people's emotions, with women showing higher scores (M=16.204, SD=2.374) [F(1,142)=4.152, p=.043]. This aligns with studies that reported that women show better emotional intelligence skills. The regression analysis verified that age (β =.-19, p=.01), self-emotion appraisal ($\beta=.28$, p=.001), and use of emotion ($\beta=.39$, p<.001) are predictors of the satisfaction with life, which explains 32% of the variance. Some studies found a negative association between life satisfaction and age. On the other hand, the increase in self-emotion appraisal and use of emotion explains higher scores of satisfaction with life, a result that aligns with previous research. The results reinforce previous studies in which emotional intelligence is directly related to life satisfaction.

Keywords: Emotional intelligence; life satisfaction; victims; non-victims.

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P3 Ensuring access to innovative, sustainable, and high-quality health care



PLGA Nanoparticles Encapsulated with Balsam Poplar Buds Aqueous Extract for Potential Ocular Delivery [†]

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Natural plant extracts are considered to be a relevant source of promising compounds, both for food and pharmaceutical industries. Poplar buds extract, widely used in folk medicine, is known for its various therapeutic properties and has shown promising health benefits, including antiinflammatory and antioxidant potential. However, the target delivery of bioactive compounds is often a limitation concerning the usage of these natural extracts. To circumvent this hurdle, PLGA (poly(lactic-co-glycolic acid)) nanoparticles have been extensively studied as drug delivery systems due to their biodegradability, biocompatibility, and controlled release properties. In this work, aqueous balsam poplar buds extract was encapsulated into PLGA nanoparticles for potential ocular delivery, applicable to ophthalmic inflammatory and oxidative processes associated with diseases and ageing. Firstly, the aqueous extract of balsam poplar buds was prepared and, then it was freezedried. For the extract encapsulation into PLGA nanoparticles, a W/O/W system was developed, based on a previously reported work by our group. As a control, unloaded nanoparticles were also prepared. Several physicochemical parameters were afterwards evaluated by dynamic light scattering. These included particle size, zeta potential, as well as polydispersity index (PDI). Then, nanoparticles were frozen at -80±1°C in order to evaluate the freezing impact and also the freeze-drying impact. The morphology of nanoparticles and lyophilized samples was evaluated by scanning electron microscopy (SEM). The encapsulation efficiency of balsam poplar buds extract in PLGA nanoparticles was 76.88±4.33. The zeta potential of unloaded nanoparticles before and after freezing was -38±0.70 and -19.80 ± 1.24 , respectively, and the size increased from 284.40 ± 24.65 nm to 408.77 ± 19.85 nm. The PDI increased from 0.315 ± 0.03 to 0.46 ± 0.02 . For nanoparticles loaded with balsam poplar buds extract, the zeta potential before and after freezing was -35.4±0.98 and -19.87±1.42, respectively, and the size increased from 276.27±9.51 nm to 419.63±46.3 nm, with a PDI increase from 0.213±0.03 to 0.45±0.02. The freeze-dried unloaded nanoparticles had a zeta potential of -23.77±1.85, size of 425.87±32.55 nm, and PDI of 0.438±0.061. The loaded nanoparticles had a zeta potential of -22.7±1.31, size of 493.77±27.91 nm, and PDI of 0.492±0.09. the unloaded nanoparticles had lyophilization ratio of 1.50, whilst the loaded nanoparticles with balsam poplar buds extract had lyophilization ratio of 1.79. SEM results indicated that the nanoparticles maintained their spherical shape after lyophilization. The freezing and freeze-drying processes had an impact on the zeta potential, size, and PDI of both unloaded and loaded nanoparticles. The lyophilization process led to the formation of a porous structure that was necessary for the resuspension of nanoparticles. These results indicate that further investigation is necessary to adapt these nanoparticles to ocular delivery systems.

Keywords: balsam poplar buds; nanoparticles; delivery systems.

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Integrative Learning in Forensic Sciences: questioned document analysis (a practical case) ⁺

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Knowledge acquisition in forensic sciences poses specific challenges that emerge from its multidisciplinary nature that overlaps knowledge from the fundamental sciences, social and legal sciences. In this context, a pedagogical proposal was address to 1st year Master students of Mestrado em Tecnologias Laboratoriais em Ciências Forenses, that aims to develop their competencies in research methodology, promote integrative learning and critical analysis throughout the application of advanced laboratory techniques for document analysis. The proposal consists in an independent protocol development to compare: a) an official document with a questioned document and b) three ink pens (1 authentic and 2 questioned) with the inks deposited in both documents. The analysis approach consisted in: 1) non-destructive techniques: Raman spectroscopy (Mira DS from Metrohm®), Reflectance Spectroscopy (Flex STD Sarspec®), optical microscopy coupled to colorimetry, different wavelength radiation (VSC 4305DMH Regula®), and physical paper characteristics such as size, color, thickness (Mitutoyo®), grammage and specific volume; 2) destructive techniques: chemical paper analyses of pH (pH pen with chlorophenol red - Abbey pH Pen^M), the type of pulp (mechanical or chemical maceration - floroglucin reagent), the fiber type (wood or non-wood fibers), ultraviolet visible (UV/Vis) spectroscopy (Flex RES+ Sarspec®) and thin layer chromatography (TLC), using silica gel plates F254 (VWR) and the eluent ethyl acetate-ethanol absolute-distilled water (70:35:30 v/v). The former techniques were preceded by constituent's extraction with 100 µL of methanol from 10 micro punches of ballpoint ink samples. Spectra obtained was optimized with Spectragryph-optical spectroscopy software V. 1.2.16.1. VSC revealed differences between the printing techniques and stamp origin of authentic and questioned documents. The ink of BIC® pen (authentic document) showed a different reaction to 505 nm radiation exposition when compared to the questioned document. As for the questioned pen samples and for the questioned document, the same response to the 505 nm exposition was observed. The Raman and reflectance spectroscopy analysis also showed dissimilarities between the spectra obtained, indicating that the stamp and ink were distinct on both documents. UV- Vis analysis revealed that BIC[®] ballpoint pen (authentic pen) resulted in different spectra and in TLC two separate spots were observed while for the questioned pens four spots were obtained for Titanium Fix® pen and three spots for the Note[®] ballpoint pen. It was also observed that both inks in questioned document (candidate's name and teacher's signature) showed three visibly separate spots. Concerning paper analysis, all the physical characteristics analyzed were similar in both documents. Students presented a written report with the obtained results and defended their conclusions. After the discussion, students were asked about the pedagogical contribution of this learning methodology to their knowledge acquisition. Positive feedback was obtained were the students involvement and the practical application were the main positive points. This integrative methodology allowed to explore different laboratory techniques in order to produce and support a scientific conclusion.

Keywords: ink; paper; microscopy; Raman, reflectance and UV/Vis spectroscopy; TLC.

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Evaluation of the morphology of the palatal rugae in Portuguese subjects⁺

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Palatal Rugae (PRs) are genetically determined, bilateral transversal elevations of variable prominences observed in the anterior region of the hard palate behind the incisive papilla. PRs exist from birth in different configurations and their structures are individual-specific like fingerprints. Furthermore, PRs patterns vary between different ethnicities. Therefore, the present retrospective cross-sectional observational study investigated the pattern and sexual dimorphism of PRs in Portuguese subjects attending the Orthodontic Clinic of Egas Moniz School of Health and Science. A total of 120 maxillary dental casts of Portuguese subjects (mean age 19.9, SD±6.7 years) were observed and categorized under adequate light and magnification according to the modified classification of Thomas and Kotze. All PRs >3mm in length were included in the study and categorized according to their morphology to: straight, wavy, curved, circular and crosslinked. Descriptive statistics, Wilcoxon signed rank, and Pearson's Chi square tests were applied to assess the prevalence, symmetry and the correlation of PRs with sex. The level of significance level was set at P<0.05. The cohort comprised 44.2% males and 55.8% females with no significant correlation between sex and prevalence of PR ($P \ge 0.372$). The total number of PRs was 624. The most prevalent PR shape was straight (44.6%), and wavy (40.7%). The occurrence of converging (11%), diverging (2.7%) and circular PR (1%) were the least observed, while cross-linked PRs were not observed. An asymmetric PR morphology was noticed only between the first paired PRs (P=0.018), while there was a similar pattern between the remaining pairs of the PR at $P \ge 0.125$. Straight and Wavy PR morphology were the most frequently observed in Portuguese individuals. There was no sexual dimorphism related to PR pattern and asymmetry was observed only among the first paired PRs.

Keywords: Orthodontic population, Palatal rugae, Portuguese individuals, Straight palatal rugae



The Impact of Incisor Molar Hypomineralisation in a Paediatric Population ⁺

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The approach to the patient with Incisor-Molar Hypomineralisation (IMH) is challenging for dentists, goes beyond overcoming the technical challenges associated with the intrinsic characteristics of hypomineralised enamel, and is recognised worldwide as a potential public health problem. The aims of this study are, quantify and assess (IMH), to understand children/young people's perceptions and individual oral health needs, to relate children/young people's perceptions to those of their parents/guardians, and finally to assess the association of the impact of IMH on children/young people's quality of life (QoL). The sample consisted of children with IMH attending the Clínica Dentária Egas Moniz (CDEM) between January and May 2022. Participants were selected based on the following inclusion criteria: individuals with at least one first permanent molar affected by IMH attending the CDEM; individuals aged between 11 and 18 years; patients with no known medical pathologies; informed consent, free, explained, and signed by the parent or legal guardian. Based on that fifty six children with IMH were observed, 27 females and 29 males, the most prevalent age was 11 years and most of them had a mild or moderate degree of the malformation. The 14-18 age group was most aware of the problem, and females were the most alert and interested. The perception of the disease in children/young people varies according to gender and age group. The impact of IMH on children's quality of life was not significant. There were no significant differences between children's responses and those of their parents/guardians

Keywords: Molar-incisor hypomineralization; perception; impact; quality of life



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Impact of a combined exercise program on healthrelated quality of life and functional capacity in post-operative lung cancer patients: a protocol for a randomized controlled trial ⁺

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Lung cancer is the leading cause of death by cancer in Portugal and is associated with significant deterioration in the quality of life of patients and poor vital prognosis. In the initial stages of the disease, surgery is a potentially curative treatment, but it is also associated to a decrease in quality of life. Studies are not clear about the impact of physical exercise on quality of life and functional capacity, and there are no specific guidelines for high intensity interval training (HIIT) in this population. A protocol for a randomized controlled trial was developed with the aim of determining the impact of a combined exercise program on the quality of life and functional capacity of operated stage I and II lung cancer patients. This randomized controlled trial follows the recommendations of Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT). Sample size was calculated using GPower 3.1. software and adjusted for a 25% dropout. A total of 40 participants are necessary. Eligible Stage I and II lung cancer post-operative patients will be randomized in a 1:1:1 ratio to the control group, combined exercise of high intensity interval training (HIIT)/resistance group and combined exercise or moderate intensity continuous training (MICT)/resistance group. Participants in both exercise groups will undertake a 12-week program with twice-weekly supervised exercise sessions and all participants will be included in the follow-up of 3-6-12 months and annually until 5 years. Patients will be assessed at baseline, upon completion of the intervention and the follow-up, by a blind assessor, for the impact of exercise on their quality of life using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core-30 (EORTC QLQ-C30), functional capacity using the 6 Minute Walk Distance (6MWD), sensation of fatigue/dyspnea (EBM) and exercise capacity using cardiorespiratory stress test. The level of statistical significance will be defined by a p < 0.05. Mixed within-between ANOVA will be used to check for significant differences in outcomes between and within groups over time. The literature is not clear regarding which exercise program is the most beneficial for this population. This study will help determine whether a combined HIIT/resistance training program can produce equivalent or even superior changes in health-related quality of life and functional capacity. If it proves effective, it will provide a strategy that can readily be implemented into clinical practice.

Keywords: lung cancer; quality of life; functional capacity; exercise



Production of leucite/zirconia dental prosthesis by robocasting with antibacterial properties[†]

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Zirconia (ZrO_2) is a highly used material for dental restorations due to its capacity to endure substantial loads during chewing without breaking. Besides, it causes low wear on antagonist dental surfaces. Zirconia is usually coated with glaze to enhance aesthetics. However, this type of coating can lead to abnormal wear on the opposing teeth due to the fragile nature of the coating. The use of glass-ceramic composites may become a good solution to overcome this issue. Subtractive manufacturing is the most used technique in dentistry, but additive manufacturing has been emerging as an alternative technology to produce dental materials. Up to date, there are some works in the literature reporting the several advantages of 3D printing, showing that dental materials with suitable properties can be successfully produced. In this work, samples of leucite reinforced with 12.5%, 25% and 37.5% (%wt.) ZrO_2 were produced by robocasting and characterized in terms of translucency, microhardness and fracture toughness. To access the tribological behavior, chewing simulation tests were performed in artificial saliva against dental human teeth. Additionally, the application of an antibacterial coating (silver diamine fluoride (SDF) + potassium iodide (KI)) over the best performing material was evaluated. The results showed that 25% ZrO₂ samples presented the highest values for both microhardness and fracture toughness. More, 25% ZrO₂ suffered almost neglectable wear and induced the lowest wear on the antagonist cusps. Compared to zirconia coated with glaze, 25% ZrO₂ led to lower cusps' wear and higher translucency. Finally, it was found that the SDF+KI coating over 25% ZrO₂ samples hampered Staphylococcus aureus adhesion and proliferation. In conclusion, robocasting can be considered a potential technique to produce leucite/zirconia materials. 25% ZrO₂ revealed to be the best performing material regarding mechanical properties (microhardness and fracture toughness), and prosthesis/cusps wear. Moreover, the SDF+KI coating applied over 25% ZrO₂ samples can help preventing complications associated with prosthetic crown placement, by impairing bacteria's attachment and growth.

Keywords: 3D printing, Leucite, zirconia, composites, rheology, tribological behaviour, antibacterial properties.

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Empowering pharmacists to generate evidence in practice ⁺

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The South and Autonomous Regions Branch of the Portuguese Pharmaceutical Society (PPS) created in 2022 the "Centre of Studies for the Pharmacy Profession", based on three domains that align with the One International Pharmaceutical Federation (FIP) concept: Education, Practice and Training. The main objective of the Centre is to share information about the value of the professional activities of pharmacists. Embedded in the "Training" domain of the Centre, PPS developed a "Scientific Capacity Building Programme" which aims to raise awareness and train pharmacists to generate and use evidence in their professional practice. This study aims to describe the development of this Programme and to share preliminary results. To set up the "Scientific Capacity Building Programme", the PPS team benchmarked other initiatives and held several meetings to discuss the content and format of the learning modules. Once structured by the PPS team, the Programme was presented to partners with the aim of involving them and receiving feedback. The Programme comprises a learning pathway divided into five modules (Awareness, Preparedness, Development, Communication and Progress) over two years. The learning modules include a diversity of formats (i.e., webinars, asynchronous and synchronous courses) that are applicable to different participants according to the level of knowledge (from basic to advanced level) with the aim of reaching all interested pharmacists (from community pharmacists to PhD students, or even academics). To date, two initiatives have been held. The first initiative was a kick-off event for the Programme and consisted of a basic level webinar on "Clinical Pharmacy Services and Evidence Generation", which was held online in March 2023. The discussion was attended by a group of 127 participants, 100% (n=127) of whom received a certificate of attendance. Following the conclusion of the webinar, a satisfaction survey was sent to participants. Among the 31 respondents, 54,84% (n=17) of them reported being "very satisfied" with the programme. The second initiative, within the preparedness learning module, consists of a basic level synchronous course that was held online in May 2023, and lasted 8 hours. The course focused on "Research in Professional Practice" and enabled the participants to plan, design and carry out research projects in a professional context. A total of 59 participants registered for the course, and 44,07% (n=26) completed it. Of the 10 participants who responded to the satisfaction survey, 90% (n=9) rated the topics as "very relevant" or "relevant". As a result of the presentation of the programme to PPS partners, four asynchronous courses are planned to be developed in collaboration with the Egas Moniz School of Health and Science. Topics concerns "Integrity in Scientific Research", "Bibliographic Research", "Overview of Science Publication" and "Visual Communication in Science". Pharmacists have shown interest on the "Scientific Capacity Building Programme". PPS considers this Programme a important step towards the development of practice-based research, thus contributing to the recognition of the impact of the pharmaceutical workforce on health systems and, ultimately, on the improvement of populations' health.

Keywords: Evidence; Pharmacists; Pharmacy Practice



Study of the biocompatibility of bioactive restorative materials $^{\rm t}$

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The rehabilitation of a tooth that is partly fractured or decayed must involve the use of a restorative material. Generally, in cases of direct restorations, the most commonly used are composite resins and glass ionomer cement. Currently, there are many resins available to rehabilitate teeth. The desire for faster and easier use leads to the development of new innovative materials on the market. In fact, the latest introduction, are the bioactive materials that will combine the strength of composites and the advantages of glass ionomer. Every year more than 500 million dental restorations are performed worldwide, with a direct or indirect interaction of the materials used on the dentin or pulp. The importance of performing biocompatibility testing is to ensure the safety of these new materials in clinical practice. New bioactive materials have appeared in recent years and, biocompatibility studies are rare or non-existent in the scientific literature. The aim of this study is to evaluate the level of cytotoxicity of different bioactive materials in 3T3 mouse fibroblasts, a very stable cell line recommended for these assays. The bioactive materials studied were Activa BioActive Restorative, which is classified as a resin-modified glass ionomer cement and Cention Forte which resembles a composite. In parallel two known and widely used restorative materials were tested as references, namely composite resin Filtek Z250 and Photac Fil Quick Aplicap which is a light-curing glass ionomer. To perform this study, the materials were prepared following strictly the manufacturer's instructions. The polymerization was performed chemically by mixing a powder with a liquid or two liquids and finalized by light-curing. The disc-shaped materials with the dimensions 4 mm x 4 mm were then placed for 24h in cell culture medium volume according to the recommendations of the ISO 10993-12 2012, standards, in order to obtain extracts. Several concentrations of 100%, 75%, 50%, 25% and 10% of the extracts were used to evaluate the effect on fibroblasts. The cell viability MTT assay was performed to determine materials cytotoxicity. Preliminary results of the MTT test show that concentrated resins extracts (100%), from Photac and Cention Forte and from 100% and 75% extracts concentrations of Activa Bioactive Restorative induce a decrease in cell viability showing cytotoxicity, which decreases with increasing dilution. Only Filtek Z 250 showed no cytotoxicity, even at the highest concentrations. We can conclude that concentrated resins extracts tested of Activa Bioactive Restorative, Photac and Cention Forte are cytotoxic probably because of the released constituents and/or changes in the medium pH and therefore they should be used with precaution in clinical oral rehabilitation. After these tests, we can also say that only Filtek Z250 has shown characteristics qualifying it as biocompatible. The fact that there is currently no data available on the citotoxicity of these bioactive materials, although this is an important factor, highlights the importance of our study, in order to achieve a high level of safety with regard to patient care.

Keywords: Cytotoxicity; restorative; bioactive; fibroblasts



Pneumomediastinum after dental procedure ⁺

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Dental manipulation is a recognized cause of cervical emphysema but rarely to pneumomediastinum. We present a case report of slow progression but extensive cervical emphysema and pneumomediastinum after a dental procedure with a bicarbonate jet in which the first symptom was right aural fullness. A 57-year-old man presents at the ENT Emergency room of with complaints of ear fullness, neck tightness, and chest discomfort. The patient reported a medical dentist appointment the previous day where he underwent a dental cleaning with a bicarbonate jet. He reported a painful event during the procedure that resolved without needing analgesics. The symptoms began hours later with aural fullness and aggravated over the night. On physical examination, the patient showed signs of cervical emphysema with subcutaneous crepitus which extended to supraclavicular and right parotid regions not evident on neck inspection. Detailed intraoral inspection showed a lesion on the sublingual space, related to the localization of the previously reported pain (documented). Laboratory findings showed leukocytosis (16 400) without other abnormal findings. A chest x-ray was performed immediately, and a Cervical computerized tomography showed signs of extensive subcutaneous emphysema on the anterior neck region with extension to the right masticatory region, retropharyngeal, parapharyngeal and anterior mediastinum spaces. The patient remained on close surveillance, during the next 48 hours, under antibiotics (amoxicillin plus clavulanic acid) with complete recovery. Neck anatomy is complex and air dissection pathways can explain the variety of signs and symptoms presented. The latter might not be evident for hours or days and prolonged surveillance is necessary. Aural fullness might be the first symptom and despite being unspecific, might prompt a proper evaluation when combined with a suggestive history.

Keywords: Pneumomediastinum; Tooth cleaning; Emphysema


L-PRF in the healing of connective tissue grafts at the donor site - protocol of a randomised clinical trial [†]

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Gingival recession results from apical displacement of the gingival margin in relation to the cementoenamel junction (CEJ), partially exposing the tooth roots. A root exposure has a number of consequences, such as hypersensitivity of the teeth, root caries and a non-harmonious aesthetic situation. Several surgical techniques are currently used for root coverage. The connective tissue graft combined with the coronal advancement flap is considered the gold standard for correction of gingival recession. The morbidity associated with the donor site of these grafts is one of the main disadvantages. Recently, L-PRF membranes have been applied to the donor area to improve the post-operative period and accelerate healing. The aim of this study is to clinically evaluate palate healing and post-operative pain. This study is being carried out at the Clínica Dentária Universitária Eqas Moniz (CDUEM). A total of 24 patients with gingival recession indicated for root coverage surgery will be enrolled. The 24 patients are equally and randomly assigned to the control group (with hemostatic sponge on the palate) and the study group (with L-PRF on the palate). Three followup visits are performed after surgery to evaluate the following parameters: quality of surgical wound healing using the Early Healing Index (EHI); perception of postoperative pain using the VAS scale; edema; hematoma; presence of suppuration; necrosis of the palate; loss of sensation and/or tissue dehiscence. These parameters and photographs of the graft donor site are taken on the day of surgery (T0), 1 week after surgery (T1), 2 weeks after surgery (T2) and 3 weeks after surgery (T3). Studies concluding that L-PRF improves the postoperative period are still limited. As the results of the study are positive, we can conclude that the use of L-PRF in the area of the surgical wound improves patient morbidity in the post-operative period, justifying its routine use in this type of surgery.

Keywords: Gingival recession; Root coverage; Connective tissue graft; L-PRF



The Link Between Intimate Partner Violence, Outness and Internalized Homophobia in an LGBT+ Sample [†]

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Intimate partner violence (IPV) occurs in couples regardless of sexuality or gender. However, research on LGBT+ couples of the same gender is still lacking. When trying to explain unique causes or links to IPV in same-gender couples in scientific literature, two variables seem to stand out in scientific research as of particular interest to IPV experiences: outness and internalized homophobia. Outness is the level of comfort an individual has in publicly assuming their attraction to the same gender, feeling comfortable with their sexuality, and being "out" to their family, friends, peers, and strangers. Internalized homophobia can be defined as a gay, lesbian, or bi person's negative and internalized feelings regarding their sexuality, or just gay, lesbian, or bisexuality in their peers, such as feeling shame over being attracted to the same gender or hating gayness, lesbianism, and bisexuality in general. Literature suggests that higher levels of outness lead to lower levels of IPV, while higher levels of internalized homophobia lead to higher levels of IPV. This study aimed to verify the prevalence of IPV among LGBT+ couples, verify the levels of outness and internalized homophobia, and explore how outness and internalized homophobia correlate with IPV. A protocol consisting of the sociodemographic questionnaire, the Internalized Homophobia Scale, the Outness Inventory, and the Conflict Tactics Scale 2 was shared online. All participants were granted their consent to participate in this study. The study followed the ethical principles outlined in the Declaration of Helsinki and was approved by the Institutional Review Board. After inclusion and exclusion criteria were applied, a sample of 48 gay, lesbian, and bisexual men, women, and nonbinary participants was obtained. Outness was positively correlated with internalized homophobia (external factor), sexual coercion, and homophobia (external factor) and negatively correlated with victimization in negotiations with a partner. When exploring the correlations between outness, internalized homophobia, and IPV, results contradicted the existing literature, as they showed that higher levels of outness lead to higher levels of IPV while finding no significant correlations between internalized homophobia and most of the CTS2's factors, which measure types and prevalence of IPV. This could be due to a smaller sample and the complexity of LGBT+ issues. Future studies should include large and diverse samples. It could also be relevant for future studies to explore the LGBT+ experience of IPV with partners of the opposite gender, which was not explored in this study. The quality of information about these types of IPV can also be enhanced using qualitative approaches, such as individual interviews.

Keywords: LGBT+; Homosexuality; Intimate Partner Violence



Effect of Different Pigment Solutions on the Microhardness of Composite Resins Materials ⁺

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Under oral conditions, composite resins may be exposed continuously or intermittently to chemical agents that may alter the surface of these materials. The wear resistance of dental materials has a significant impact on the clinical success rate of restorations. The purpose of this study was to evaluate the microhardness of a microhybrid composite resin and a bulk-fill composite resin after immersion in different pigment solutions. 130 samples of Filtek® Z250 and Filtek® One Bulk Fill, 3M® ESPE (Saint Paul, MN, USA) composite resins were prepared in stainless steel (5x2 mm) cylindrical molds according to ISO 4049:2019. The samples were photopolymerized for 20 seconds and then randomly divided into experimental groups, accounting for two immersion procedures (continuous and discontinuous) and six immersion solutions (n=5): artificial saliva, coca-cola[®]; natural lemon juice, aloe vera juice, red wine, 96% alcohol and control group. In the continuous immersion test, the samples were continuously immersed in the solutions for 7 days, and, in the discontinuous immersion test, the samples were immersed for 30 minutes, 3 times a day for 7 days. Between immersion periods the samples were stored in artificial saliva. The Vickers microhardness (5 s, 9.92 N) was measured before and after the 7 days of immersion. Inferential statistical analysis was performed using a three-way ANOVA, at a 5% significance level, considering the factors (immersion procedure, immersion solution and composite resin type). The results showed statistically significant differences in microhardness, attributed to all three factors: immersion procedure, immersion solution and resin type (p < 0.001), with the immersion solution being identified as the most pronounced effect. Alcohol was the solution that least decreased the microhardness of the composite resins followed by red wine, natural lemon juice, coca-cola®, aloe vera and artificial saliva. Filtek® Z250 is the material that has undergone the least change and has retained the most of its surface microhardness (p < 0.001). The discontinuous immersion method contributed to a lower mean microhardness variation compared to the continuous method (p < 0.001).

Keywords: Composite Resins; Bulk Fill; Microhardness Vickers; Continuous/Discontinuous Immersion



Mental Representation of the Smile in Adults Patients undergoing Orthodontic Treatment⁺

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Previously, in the last five years, studies were carried in Egas Moniz University Clinic surrounding the mental representation of the smile, centred in adolescent's patients wearing orthodontic appliance, guided by the need of emerge, in the line of the results obtained, a new form of categorizing the perfect smile - The Orthodontic Smile. The present study is qualitative and exploratory, and aims to understand the importance of the self-perception (mental representation) of the oral cavity and the orthodontic smile, now with the help of a new age range – Adult Patients. The sample of the present study consists of 100 subjects in the adult phase (25-59 years old), namely subjects wearing orthodontic appliance who were asked to draw two percepts about the selfperception of their own smile, in two distinct moments: The first drawing, corresponding to before the wear of the orthodontic appliance (M1), and, the second one, the patients were asked to draw a percept of their smile after undergoing the orthodontic treatment (M2). The content analysis of the 200 drawing obtained, was realized through an analytical grid of analysis content, constituted by categories and subcategories. The patients also filled a socio-demographic questionnaire, in which they responded to 4 open ended questions, in relation to the mental representation of the smile: i) What is considered for you as a nice smile? ii) Would you change something about your smile? iii) What do you think now about your orthodontic smile? iv) Why did you seek dental treatment? A content analysis of the open ended questions was also performed, by using a content analysis grid of the narrative of the responses from the subjects, especially created for this study. Regarding the analysis content of the narrative of the open ended responses, we highlight the fact that a Nice Smile, seem to be, mostly described as a Natural Smile and Aligned, being a motive to the seek of dental treatment, primarily aesthetic issues, followed by functional considerations. When comparing the drawings before (M1) and (M2) the wear of orthodontic appliance, we note that the Category Broad Smile, is set up as the smile chosen as the most aesthetic. It seems that aesthetics motives represent the motivational leverage for the patient in the adult phase, when they decide to look for Orthodontic treatment.

Keywords: Mental Representation; Orthodontic treatment; Aesthetic Smile; Self-perception of the Smile; Orthodontic Smile; Adult Phase



Ag(I) camphorimine complex eluting dressings for malignant fungating wounds ⁺

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Malignant fungating wounds (MFW) derived from the tumour or metastasis infiltration into the skin, afferent blood and/or lymph vessels are generally painful and give rise to high levels of exudate, bleeding and malodour. The management of MFW may involve surgery, radio-, electro- or chemotherapy, which are known to cause adverse side effects (e.g., fatigue, pain, gastrointestinal problems, sores). Daily care relies on the application of wound dressings to control exudate and bleeding, and on topical administration of antiseptics and anti-inflammatory drugs. However, the currently available dressings do not answer to the specific conditions and needs of MFW. It is imperative to prevent and minimise wound enlargement, infection, periwound skin breakdown, reduce pain, and ultimately reduce the patient' overall suffering. In this work, an 2-hydroxyethyl methacrylate (HEMA)-based hydrogel was synthesised and loaded by simple soaking at room temperature with the silver camphorimine complex [Ag(NO3)(OC10H14NNH2)] (JP350B) and sterilised by a lower thermal input method, high hydrostatic pressure (HHP, 600 MPa, 10 min, 70 °C). The used drug presents antimicrobial and anticancer activity, and therefore may enable a more effective treatment of skin wounds in cancer patients. In fact, these types of wounds are more prone to infection by bacteria or fungi due to the individual's depleted immune systems. Material's properties relevant for their performance in wound dressings were studied, namely wettability, ionic and O2 permeability and swelling. Tensile tests and rheological tests were also carried out to evaluate their mechanical resistance. Drug release experiments were performed in sink conditions. The antimicrobial and anticancer activities of JP350B were assayed as previously described. Finally, chorioallantoic membrane (HET-CAM) tests were done to infer about potential tissue irritation. The produced HEMA-based hydrogel showed suitable physico-chemical properties to be used in MFW dressings and was able to ensure a controlled release of JP350B for at least 2 days. Sterilisation slightly affected the drug loaded system, namely the drug release profile and therapeutic properties of the drug, but overall, the properties and the materials behaviour remained adequate for the intended application. JP350B showed cytotoxic activity against both A375 and G361 human melanoma cells and antimicrobial activity against e.g., P. aeruginosa. HET-CAM tests suggested that HEMA samples should not induce tissue irritation. In conclusion, the HHP sterilised HEMA-based hydrogel loaded with a silver camphorimine complex seems to be promising to obtain therapeutic dressings, with efficient anticancer and antibacterial response.

Keywords: Silver camphorimine complex, Hydrogels, Drug release systems, malignant fungating wounds, Antibacterial, Anticancer

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Demographic changes in a large motor neuron disease cohort in Portugal: a 27 year experience ⁺

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Motor Neuron Diseases (MND) have a large clinical spectrum, being the most common amyotrophic lateral sclerosis (ALS) but there is significant clinical heterogeneity. Our goal was to investigate this heterogeneity and any potential changes during a long period. We performed a retrospective cohort study among a large Portuguese cohort of MND patients (n=1550) and investigated changing patterns in clinical and demographic characteristics over the 27-year period of our database. With that aim, patients were divided into three 9-year groups according to the date of their first visit to our unit: P1, 1994-2002; P2, 2003-2011; P3, 2012-2020. The overall cohort's clinical and demographic characteristics are consistent with clinical experience, but our findings point to gradual changes over time. Time pattern analysis revealed statistically significant differences in the distribution of clinical phenotypes, average age of onset, diagnostic delay, proportion of patients using respiratory support with non-invasive ventilation (NIV), time to NIV, and survival. Across time, in the overall cohort, we found an increasing age at onset (p=0.029), a decrease of two months in diagnostic delay (p<0.001) and a higher relative frequency of progressive muscular atrophy patients. For ALS patients with spinal onset, from P1 to P2, there was a more widespread (54.8% vs 69.4%, p=0.005) and earlier (36.9 vs 27.2 months, p=0.05) use of NIV and a noteworthy 13 month increase in median survival (p=0.041). Our results probably reflect better comprehensive care, and they are relevant for future studies exploring the impact of new treatments in ALS patients.

Keywords: amyotrophic lateral sclerosis; epidemiology; phenotype; prognosis; temporal changes.

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Rapid tests for Carbapenemases detection among Enterobacterales clinical strains ⁺

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Increasing carbapenem resistance in Enterobacterales (CPE) represents a major public health threat. The spread of antimicrobial resistance in Gram-negative bacteria is increasingly limiting therapeutic options. Infections caused by CPE are associated with limited therapeutic options and high mortality rates. Rapid tests have recently revolutionized the problem of early diagnosis due to their accessibility and speed of results. Their accurate and rapid detection of carbapenemase producers and determination of the class of carbapenemases according to Ambler's classification allow to guide antimicrobial therapy and facilitate infection control measures. The aim of the study is to determine the sensitivity and specificity of the immunochromatographic assay NG-Test CARBA 5 (NG-Biotech, France) compared with the Polymerase Chain Reaction standard test (PCR). Thus, it was performed an experimental study with Enterobacterales clinical strains (n=141) recovered from three hospitals in Portugal. The strains belong to six different species: Klebsiella pneumoniae (n=125), Enterobacter cloacae (n=7), Enterobacter aerogenes (n=1), Klebsiella aerogenes (n=6), Klebsiella oxytoca (n=1) and Citrobacter spp. (n=1). The multiplex results of the rapid tests were compared with the standard PCR test. The NG-Test CARBA 5 assay allows the qualitative and differential detection of 5 common carbapenemases: NDM, KPC, OXA-48, IMP, and VIM. This study has demonstrated a rate of 97.3% sensitivity and 100% specificity, while the manufacturer reported sensitivity and specificity of the test is 100%. It is important to note that a positive or negative result of the NG-Test CARBA 5 does not exclude the presence of other antibiotic resistance mechanisms and therefore requires further studies. Moreover, the conflicting results were obtained on the detection of OXA-181 and KPC-3 new variants. Furthermore, it is necessary to consider further studies in this thematic to promote an accurate and early detection of antimicrobial resistant strains.

Keywords: Carbapenemases; Rapid tests; Enterobacterales



Schneiderian membrane perforation rate during sinus floor elevation with osseodensification – a retrospective study [†]

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Sinus floor elevation (SFE) by crestal approach with osseodensification (OD) is a predictable and safe treatment alternative when residual bone height (RBH) is insufficient for conventional implant placement. The purpose of this retrospective study was to assess the incidence of Schneiderian membrane perforation with OD technique. Patients who were consecutively treated with OD for SFE and simultaneous implant placement between October 2020 and September 2022 were included in this retrospective study. All cases were either single-unit implants or short-span bridges with 2 to 3 elements. Timing of perforation detection was recorded as either intraoperative or postoperative if it was only identified on the final radiograph. In total, 67 patients with 78 implants were included in the final analysis. Mean residual bone height at baseline was 5.05 mm (range from 1.5 mm-8 mm). Six perforations were detected (three intraoperatively and three postoperatively) in the 78 sinus lifts for an overall incidence of Schneiderian membrane perforation of 7.7%. The perforations identified intraoperatively were managed with the placement of an implant shorter than initially planned. Three perforations were only identified in the postoperative periapical radiograph since the grafting material was not fully contained. None of the perforations led to postoperative infection or implant failure. This retrospective study showed that OD is a predictable and effective technique for SFE by crestal approach with simultaneous implant placement. Incidence of Schneiderian membrane perforation was lower compared to perforation rate reported in the literature for the classical lateral window approach.

Keywords: osseodensification; sinus floor elevation; implant placement; membrane perforation.



Inflammatory effects of nanoplastics and interaction with benzo[a]pyrene in human colon cells ⁺

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The presence of nanoplastics (NP) in the environment and their potential impact on human health have raised concerns in recent years. These smaller plastic particles exhibit unique characteristics, including increased reactivity and the ability to adsorb significant amounts of other harmful pollutants. In this study, we aimed to investigate the inflammatory responses induced by NP, specifically their interference with inflammation-related pathways in HT29 human colon adenocarcinoma cell line. Furthermore, we assessed the effects of co-exposure to NP and benzo[a]pyrene (B[a]P), focusing on biomarkers associated with the detoxification mechanisms of B[a]P, such as 7-ethoxyresorufin O-deethylase (EROD), glutathione-S-Transferase (GST) activity and glutathione levels (reduced (GSH) and oxidized (GSSG) forms) in cellular lysates. Cell viability was evaluated following exposure 24, 48 and 72 hours to polystyrene NP with diameters of 25, 50 and 100 nm (at concentrations of 25, 50, 100, 200 and 500 µg.ml-1), to 5 and 50 µM concentrations of B[a]P and to their mixtures (500 µg.ml-1 of 25 nm polystyrene NP + B[a]P at 5 and 50 uM). In cells exposed alone to NP, we also analyzed the p50 NF-kB and p38 MAPK pathways in nuclear fractions using Western Blot. Transcript (mRNA) levels of interleukin-1 β (IL-1 β), inducible nitric oxide synthase (iNOS) and tumor necrosis factor-a (TNF-a) were analyzed by qRT-PCR, including in cells preexposed to TLR4 and p38 inhibitors. Results revealed that cell viability was significantly affected by B[a]P exposure compared to NP, particularly after 48 and 72 hours. Exposure to smaller NP resulted in increased nuclear translocation of p50 and p38, suggesting their involvement in inflammatory signaling pathways. The enhanced nuclear translocation of p50 was associated with increased transcription of IL-1 β , iNOS, and TNF-a. Furthermore, we observed an up-regulation of TLR4 expression in cells exposed to NP, indicating the involvement of the TLR4 pathway in NP-induced inflammation. Indeed, inhibition of TLR4 attenuated the pro-inflammatory response of cells to NP, suggesting its contribution to the observed effects. Exposure of cells to B[a]P enhanced EROD activity, whereas co-exposure to NP and B[a]P led to a notable decrease in EROD activity, indicating a reduction in CYP1A1 activity and potentially lower formation of B[a]P metabolites. GST activity was found to be higher in cells exposed to B[a]P, whereas its activity decreased when co-exposed with NP, possibly due to reduced availability of B[a]P due to its absorption to NP. Additionally, our results showed that GSH synthesis may be up-regulated in the presence of NP and B[a]P co-exposure. Overall, our findings demonstrate that NP can act as a stressor of innate immunity and stimulate the inflammatory process, particularly through the TLR4 and p38 pathways. The co-exposure of NP and B[a]P affected the activities of EROD, GST, and the synthesis of GSH, indicating potential interactions and alterations in the cellular detoxification mechanisms. These findings contribute to a better understanding of the effects and toxicity mechanisms of NP and of the potential interactions with other pollutants, highlighting the importance of considering co-exposure scenarios when assessing the environmental and health impacts of these pollutants.

Keywords: Keywords: nanoplastics; benzo(a)pyrene; inflammation; genotoxicity; inflammatory pathways

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Assessment of the level of knowledge of health care professionals in Portugal regarding pain and TMD ⁺

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Oral and maxillofacial pain affects approximately 10-26% of the adult population and up to 50% of the elderly. Temporomandibular disorders (TMDs) encompass a variety of disorders of the masticatory system that can cause pain and disability affecting daily activities, psychosocial functioning, and quality of life. The assessment, diagnosis and treatment of orofacial pain is complex and requires a multidisciplinary approach, so it is important to ensure that all healthcare professionals can correctly identify, diagnose and manage patients with signs of TMD or orofacial pain. The aim of this study was to assess knowledge of pain and its relationship to TMD management among dental, medical, physiotherapy and speech therapy professionals using the Neurophysiology of Pain Questionnaire. The sample consisted of four different groups of health professionals (dentists, doctors, physiotherapists and speech therapists). Professionals completed a questionnaire consisting of Part A, which included socio-demographic questions such as age, profession, year of graduation, knowledge and assessment of TMD, and Part B, where the Neurophysiology of Pain Questionnaire (NPQ), translated and validated for Portuguese, was applied. This study was approved by the Ethics Committee of Egas Moniz School of Health and Science, Almada, Portugal on the 22nd of February 2023. For statistical analysis, the data obtained were calculated and subjected to simple quantification (descriptive) statistical analysis. In this preliminary study, due to the lack of answers from doctors and speech therapists we will only use data from dentists and physiotherapists. Of the 113 health professionals analysed, 48 had a bachelor's degree, 61 a master's degree and 4 a doctorate. 65 were dentists, 8 were doctors, 28 were physiotherapists and 9 were speech therapists. The most common response regarding frequency of diagnosis for dentists concerning TMD and/or orofacial pain was 1 to 3 times per week (26%) followed by 1 to 3 times a month (25%), physiotherapists showed that most never diagnose (32%) or when they do, it's less than once a month (29%). As for treatment, the most common answer for dentists was less than once a month (31%) followed by those who never treat it (23%), most physiotherapists said they never treat (43%) or that it happens less than once a month (21%). The results showed that the average score on the NPO for these two groups of health professionals was 8.80 out of 12, with dentists scoring 8.51 and physiotherapists scoring an average of 9,46 out of 12. The average scores for the NPQ were satisfactory, with higher scores for physiotherapists. Most dentists were likely to be involved in diagnosis more often, whereas most physiotherapists rarely did. Regarding treatment, it was shown that most physiotherapists rarely treat patients with TMD or orofacial pain, and that most dentists treat it less than once a month or never, showing that TMD and orofacial pain are not common practices among these health professionals, despite the high scores in the NPQ. A clear limitation of the study is the lack of responses from doctors and speech therapists, which we will focus on as the study continues.

Keywords: orofacial pain; TMD; healthcare professionals; neurophysiological pain questionnaire



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Characterization of the Community Pharmacies' Individualized Medication Preparation service delivered for nursing homes: a cross-sectional analysis [†]

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Multimorbidity and polypharmacy are two major challenges for healthcare professionals in their clinical practice, considering the potential to affect the patients' clinical outcomes. Previous research has shown that multimorbidity and polypharmacy can increase the risk of using potentially inappropriate medications and, consequently, the adverse drug reactions (ADRs). One of the strategies that have been implemented in the community pharmacy is the individualized medication preparation (PIM) service which helps organize patients' medications, increasing not only medication adherence, but also the active screening for drug-related problems (using medication review – MR). This study aims to characterize the PIM service provided by community pharmacies to nursing homes and evaluate the barriers to integrating RM in the service. We conducted a cross-sectional study, since April 2023, aiming to reach a theoretical sample of 202 pharmacies in Portugal. Questionnaires were sent via mailmarketing and social media platforms such as Mailchimp, Facebook, Linkedin and Instagram. Reminders were sent every two weeks. The e-guestionnaire intended to characterize the participating pharmacies, the PIM service, particularly when delivered to nursing homes, and the barriers to integrating MR into PIM. We have already collected preliminary data, which were analyzed using descriptive statistics (IBM SPSS v.19.0). Thus far, we have received 86 responses to the equestionnaires, representing approximately 43.0% of the theoretical sample. Most of the participating pharmacies were located in the Lisbon metropolitan area (35.8%; n=29) and central region of Portugal (32.1%; n=26). The mean age of team members was 37 ± 5.19 years. We obtained 39.5% (n=32) of responses from the Urban area, followed closely by the Rural area with 33.3% (n=27), and the Suburban area with 27.2% (n=22). On average, each pharmacy has approximately a total of 8 ± 4.49 employees, with the majority being pharmacists, and 43.2% (n=35) having at least one specialist pharmacist in community pharmacy. The majority of participating pharmacies provided the PIM service (75.3%; n=61), and among those, 25.6% (n=42) delivered the service to nursing homes. Out of these 42 pharmacies, 64.3% (n=27) offered the PIM service combined with MR benefiting an average of 53.1±56.7 patients. Both services were provided by an average of 2.6±0,9 employees, mostly pharmacists. A significant proportion of these pharmacies frequently monitored the need for the rapeutic changes (85.2%; n=23), using telephone (81.5%; n=22) or e-mail (74.1%; n=20). However, only 21% (n=17) performed medication adherence monitoring. The main barriers identified were the lack of recognition and visibility of the services (21.9%), insufficient collaboration from other healthcare professionals (21.1%), and lack of patient interest (16.2%). In conclusion, most pharmacies offer the PIM service, but only a quarter of them provide this service to nursing homes. The majority of these pharmacies supplement this service with MR. Addressing potential barriers identified in this study is crucial to enhance the delivery and impact of MR and PIM services in community pharmacies.

Keywords: Elderly; Nursing Homes, Medication Review; Individualized Medication Preparation; Community Pharmacies



Knowledge and practice of healthcare professionals on topical corticosteroids at community pharmacies: a cross-sectional analysis⁺

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Several studies have shown that patients are not properly advised on how to use topical corticosteroids (TCS), and this seems to be related to poor information delivered by healthcare professionals and/or a lack of knowledge on this topic. This may lead to the development of corticophobia, both in patients and healthcare professionals, affecting patients' adherence to TCS and resulting in treatment failure. The main goal of this study was to characterize the knowledge and practice of health professionals on TCS in community pharmacies. We are undertaking a nationwide cross-sectional study since April 2023, targeting all healthcare professionals working at community pharmacies, who dispense medications either independently or under the supervision of a pharmacist (pharmacy technicians and pharmacy assistant technicians). Participants were invited via email using MailChimp, and reminders were sent every two weeks. The e-questionnaire was made available on different social media platforms (Facebook, Instagram, LinkedIn), and participants were asked to answer and share it with other colleagues or on their personal social media. The equestionnaire included three sections: one for the characterization of participants' sociodemographic variables, and the other two intended to characterize their knowledge (perceived knowledge was assessed using a 5-item Likert scale) and practice (using the same scale). Data were analyzed using descriptive statistics (IBM SPSS v.19.0). So far, we have had 107 participants, of whom 86% (n=92) were female, with a mean age of 37.9 ± 9.0 years. Most of them were pharmacists (87.9%; n=94), and 44.9% (n=48) practiced in the Lisbon Metropolitan Area. The mean years of work experience were 12 ± 8.9 years. Most of the healthcare professionals felt confident in their knowledge regarding TCS, but there were some domains where a small portion of the sample felt less confident: 14% (n=15) did not feel confident when it came to either the potency of action or identifying systemic adverse effects of TCS. In their daily practice, 76% (n=81) felt that there were barriers during counseling on TCS, with the most common ones being a lack of counseling/information/educational materials (46.7%; n=50), patients' negative feelings towards TCS use (34.6%; n=37), and the assumption that patients already know how to use TCS (20.6%; n=22). In daily practice, nearly a quarter of the sample (22.4%; n=24) rarely monitored adverse drug reactions related to the use of TCS. The comparative analysis between pharmacists and pharmacy technicians was not performed due to the discrepancy between the groups. Overall, healthcare professionals from community pharmacies felt confident in counseling the use of TCS, but a small portion of them do not fully understand the different potencies of TCS and have some limitations concerning the identification of systemic adverse drug events related to TCS. In their practice, most of the participants do not monitor adverse drug events related to TCS. Future research should include the design and development of a more tailored educational intervention targeting healthcare professionals working in community pharmacies.

Keywords: Topical costicosteroids, community pharmacies, corticosphobia, counseling,



Study Protocol - Evaluation of different periodontal plastic surgery techniques for gummy smile treatment ⁺

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A smile with more than two millimeters of exposed gingiva is considered a gummy smile (GS). Usually reduces self-confidence as it affects the aesthetic and psychological status. GS has a prevalence of 10,5%-29% in the population, being more frequent in female patients. The etiology of GS may include altered passive eruption, dentoalveolar extrusion, vertical maxillary excess, short or hyperactive upper lip or a combination. Recently, there have been a number of articles describing a new technique for treating upper lip hypermobility: the implantation of a customized device in the anterior maxilla to decrease the movement of the upper lip muscles. This study aims to evaluate the gummy smile reduction and the impact on aesthetic parameters obtained after implantation of PEEK devices in the anterior area of the maxilla compared to flap surgery and gingivectomy techniques. 30 participants will be distributed equally between three groups: 1) patients with upper lip hypermobility – will receive surgery for device implantation in the anterior area of the maxilla; 2) patients with short teeth and upper lip hypermobility - will be submitted to flap surgery/gingivectomy device implantation; 3) patients who refuse implantation – will undergo flap plus surgery/gingivectomy alone. Participants will be recruited at Egas Moniz University Clinic (Almada, Portugal). After acceptance of participation in the study and signing of an informed consent, patients will undergo a preoperative clinical evaluation and then a follow-up at two weeks, one month, three months and six months after surgery. Informed consent was approved by the Ethics Committee (protocol code 1090). The primary outcome measure is: gingival exposures during a forced smile. The secondary outcome measures are: superior incisors proportion; exposure of central incisors at rest; exposure of central incisors during a forced smile; interlabial space at rest; periodontal probing; amount of keratinized tissue; gingival exposure at rest. The inclusion criteria are: adults between 18 and 45 years old; gummy smile due to altered passive eruption and/or lip hypermobility. The exclusion criteria are: smokers; pregnant or breastfeeding patients; systemic diseases that can affect the result of surgical treatment; cleft lip; craniofacial syndromes; facial surgeries during the study. Currently, there are only three articles with case reports describing this technique with polymethylmethacrylate (PMMA) devices. All from Brazil, these articles have been published between 2018 and 2023. In this study, we choose to use PEEK. Follow-up of patients over six months will allow the stabilization of all parameters to be assessed. This is something that has not been done before. It is expected that a new door will be opened for the treatment of gummy smile, which will certainly bring added value for patients and clinicians who will be able to treat their patients in a new way

Keywords: gummy smile; lip hypermobility; periodontal surgery techniques



P4 Unlocking the full potential of new tools, technologies and digital solutions for a healthy society



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Measurement stability and intersession reliability of H-reflex recruitment curve parameters using different number of stimulations per intensity [†]

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The extraction of the full Hoffmann (H-) recruitment curve represents the gold standard in human motor control and clinical neurophysiology investigations for detecting the effects of specific interventions on H-reflex circuitry. However, there is still no general agreement on how many stimulations per intensity should be applied to extract these data (only that several stimulations should be performed). Thus, we explored the impact of varying the number of stimulations per current intensity on the H-reflex recruitment curve parameters extraction. We also determined whether the intersession reliability of the H-reflex parameters varied with the number of stimulations applied per current intensity. Twenty healthy participants (11 males, 9 females; age: 22.3 ± 2.3 years) visited the laboratory on 2 separate days. In a sitting position, with participants performing a low-level plantarflexion contraction (10% of maximal voluntary isometric contraction), soleus M- and H-recruitment curves were constructed using 16 stimulations (1-ms rectangular pulses) per current intensity (22 intensities), delivered randomly at 3-s intervals. The recruitment curve parameters were then determined based on 3, 6, 9, 12 and 15 stimulations per intensity. Intrasession measurement variability of the parameters was assessed using a 2-way ANOVA with repeated measures. Test-retest reliability was assessed using the intraclass correlation coefficients (ICCs) and the coefficients of variation (CVs). The results from the ANOVA indicated that maximal M wave amplitude (p = 0.093) and H-reflex current (p = 0.299) values are precisely determined using 3 stimulations per intensity. Conversely, for the slope of the H-reflex ascending limb (p < 0.021), maximal amplitude (p < 0.026) and threshold current (p < 0.039), a minimum of 9 stimulations are required to achieve stability of the measurements (p > 0.05). The ICCs of the maximal amplitude of M wave, H wave and threshold current ranged from 0.73-0.90. For the H-reflex slope, the ICC ranged from 0.56-0.77, and for the maximal-H reflex current, it ranged from 0.25-0.49. Nevertheless, the ICCs increased progressively and stabilized at 6 and at 9 stimulations for maximal-H reflex current (0.25-0.43) and H-reflex slope (0.56-0.72), respectively. With the exception of the maximal-H reflex current (exhibiting a higher CV with 3 stimulations, p < 0.028), the CVs remained unchanged (6-32%), irrespectively of the number of stimulations (p > 0.05). Our findings indicate that, overall, a minimum of 9 stimulations per intensity segment are required to ensure maximal stability for each intrasession extraction and intersession reliability of H-reflex parameters, hence elucidating genuine changes in the reflex circuitry after experimental interventions.

Keywords: soleus, hoffman reflex, recruitment curve, evoked spinal response, electrical stimulation



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Pilot study: Assessing MMP-8 levels as a measure of periodontal disease in a Portuguese population⁺

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Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes with diverse substrates responsible for the degradation of structural components of the extracellular matrix, such as receptors, cytokines, and growth factors in many physiological and pathological processes. MMPs are expressed in several cell types, including neutrophils and macrophages, as non-active proenzymes that can selectively be activated in situ. Overexpression or deregulation of the MMPs activity is a recognised cause of several pathologies. The tissue destruction observed in periodontal disease has been attributed to increased levels and activity of MMPs in oral fluids during inflammation, with MMP-8 often cited as playing an active role. We aim to quantify the levels of MMP-8 in the periodontal pocket to determine its contribution to this disease progression in a Portuguese population. The study was approved by the Egas Moniz Ethics Committee. We collected gingival crevicular fluid (GCF) with paper strips from 24 patients with periodontitis and 21 healthy subjects, measured the volume with the Periotron analyser and determined the level of MMP-8 protein using an ELISA kit. Our results showed no significant differences in total MMP-8 concentration in GCF between the control $(3.93 \pm$ 2.8 μ g/mL) and periodontitis group (4.73 ± 2.8 μ g/mL). To analyse MMP-8 levels, independently of the GCF volume, MMP-8 levels were normalised with total protein concentration in the same sample. The relative abundance of MMP-8 thus calculated confirmed the previous results and showed no significant differences between the two groups. Although many studies, such as this one, quantify total MMP-8, this does not distinguish the fraction capable of degrading fibrillar collagen while destroying the periodontium. It was concluded that the amount of MMP-8 protein is not a good indicator of periodontal status.

Keywords: MMP-8; Periodontitis, gingival fluid

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In vitro study of the antibacterial effect of ozone on cariogenic bacteria[†]

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Ozone has been increasingly used as a complementary and conservative treatment for dental caries due to its antibacterial action. However, there is a lack of evidence and consensus on protocols. Therefore, this study aims to evaluate in vitro the antibacterial capacity of ozone on cariogenic bacteria, namely Streptococcus mutans, Streptococcus sobrinus, Lactobacillus casei and Actinomyces naeslundii (reference strains), which are the main etiological agents of dental caries, and contribute to the development of a universal protocol. Suspensions of the bacteria mentioned above were exposed to different concentrations (40, 60, and 80 µg) and contact times (40 and 80 seconds) of pure gaseous ozone and ozonated water through an ozone generator machine "Ozonette" (Sedecal, Madrid, Spain). A negative control group (where no ozone was applied) and a positive control group (chlorhexidine 2%) were used. After the ozone/chlorhexidine's action on the suspensions, aliquots were inoculated on specific culture medium and placed in the incubator at 37°C/48h. The capacity to inhibit the microorganisms tested was determined by counting the number of CFU (colony forming unit) on the inoculated media. It was observed significant bacterial reduction after ozone exposure in all the microorganisms tested, both on gaseous ozone and ozonated water groups. In conclusion, the results suggest that ozone therapy exhibits antibacterial activity against certain cariogenic bacteria, warranting further investigation as a potential preventive therapy for dental caries or as an antibacterial agent after mechanical caries elimination. However, further studies are needed to verify the safety and efficacy of ozone application in a clinical setting and to test the influence of ozone on enamel and dentin adhesion.

Keywords: Ozone; dental caries; cariogenic bacteria.

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Looking for a genetic link between systemic lupus erythematosus and the periodontal disease ⁺

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Systemic lupus erythematosus (SLE) is a systemic autoimmune condition related to dental medicine to varying extents due to its implications for different oral structures. Patients with active SLE have worse oral health, including an increased prevalence of xerostomia, mucositis, erythematosus ulcers, glossitis, angular cheilitis, mucous ulceration, temporomandibular joint dysfunction, and periodontal disease. Periodontal disease is a family of inflammatory conditions that affect the supporting tissues of teeth and may share with SLE a common immunological mechanism influenced by genetic risk factors. This study evaluated the association between SLE and periodontal disease using Mendelian randomization and protein interaction tools. The MrBase app was used to perform Mendelian randomization analysis using data from the Catalog of the National Institute for Human Genome Research – European Institute for Bioinformatics and Studies of the Human Genome Association. The DisGeNET and the Comparative Toxicogenomics Database platforms were used to pool genes related to each disease and identify which were related to both diseases. The proteins expressed by the identified genes were linked in a protein-protein interaction network built on the STRING platform. Mendelian randomization showed a weak positive effect, but without statistical significance, on the relationship between SLE and periodontal disease, MR Egger (B=0.271 SE= 0.157, p= 0.098). The protein interaction network analysis showed possible pathways between SLE and periodontal disease. The genes identified in the protein-protein interaction network related to both diseases included the FcyRs, MMPs, IL-6 and IRF5 variants, and were strictly related to acute periodontitis cases. These pathways should be studied further to assess better the relationship between these conditions.

Keywords: Periodontal disease; systemic lupus erythematosus; Mendelian randomization; protein-protein interaction network

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The influence of transcutaneous auricular electrical stimulation of the vagus nerve on anxiety and masticatory muscle activity [†]

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According to the American Psychiatric Association (2020), anxiety is defined as the anticipation of a future threat, different from fear, which evokes a real or perceived imminent response that influences the cognitive aspects of apprehensive expectation. It is one of the main psychopathologies diagnosed in university students, being justified by excessive obligations and academic concerns, and may be related to parafunctional habits, such as tooth clenching (awake bruxism), which can lead to hyperactivity of the masseter muscle. Transcutaneous auricular electrical stimulation of the vagus nerve (TaVNS), applied to the left pinna, is a therapeutic resource that has a modulating effect on nociception for subjective responses such as anxiety. This study aimed to identify the influence of TaVNS on anxiety and masseter muscle activity in university students, through electromyography (EMG), before and after the evaluation period. This study was approved by the Comissão de Ética Egas Moniz on March 2, 2023. It is a controlled, randomized, double-blind clinical trial, where participants were randomly distributed into 2 groups, the experimental (G1) and the control (G2) . After recruitment and signing of the informed consent form, data for the study were collected at 2 different times, T0 before the intervention, T1 shortly after the end of the intervention week and following the recommendations of CONSORT/2010. At each of the moments, an electromyographic evaluation of the masseter muscles at rest was performed and the self-questionnaire Back Anxiety Index (BAI), used to measure the severity of the individual's anxiety, was completed. The intervention protocol consisted of 3 sessions of 20 minutes each, during the academic evaluation period that took place between April 17th and 21st. After the end of the evaluations, the statistical analysis was obtained by performing the T-Test. It was possible to observe that G1 showed a significant improvement in anxiety levels (difference in mean scores of 3.14, p=0.009, with an effect size of 1.05, or very large), and also showed a reduction in electromyographic activation at rest after treatment (p < 0.05) for the right masseter muscle (difference in RMS means of 39.01, p = 0.011, with effect size 0.60 or moderate) and for the left masseter muscle (difference in RMS means of 26.19, p = 0.010, with a size effect of 0.33 or moderate). G2 showed no significant improvement in anxiety (difference in mean scores of 1.68, p=0.099) and showed a slight reduction in EMG activation at rest after treatment (p < 0.05) for both right masseters (difference in RMS means of 13.53, p=0.048, with effect size 0.45 or small) and left masseter (difference in RMS means of 14.87, p=0.048, with effect size 0, 14 or small). The TaVNS protocol used was effective in reducing anxiety, but it was not possible to state that there is interference in the reduction of EMG activation, as the groups were evaluated separately before and after the intervention. It is suggested that further studies be carried out to better identify this relationship.

Keywords: anxiety, transcutaneous auricular electrical stimulation, vagus nerve, neuromodulation, EMG

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Sexual Trafficking: Awareness campaign ⁺

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People trafficking is considered the third most profitable business in the world, only overpast by drug and weapon trafficking, in which human beings are considered an object. In this business, people can be trafficked for several purposes, such as sex trafficking, slavery, illegal adoption, and organ removal. Regarding sex trafficking, the goal is to profit from selling human beings into prostitution, pornography, and sexual slavery. Regarding sexual exploitation, most victims revolve around women and little girls. Nowadays, social media are being used as a recruitment tool for human trafficking. According to the Human Trafficking Federal Report, 50% of this type of victims were recruited online, almost half of whom were recruited via Facebook (41%). Recruiters entice victims through job promises, romantic relationships and/or a promise of shelter. To fulfil our goal of raising awareness surrounding sexual trafficking and the role of social media in recruiting people into this business, we made a video which exemplifies how easy it is to be recruited online and shows some of the consequences of being trafficked. To complement the video, we also made a flyer which contains some tips and strategies to guarantee the safe use of social media. Our goal is to enlighten parents on preventing this phenomenon from happening to their children or close family and friends. Our campaign consists of a brief video of a girl meeting a guy through Facebook and arranging to meet each other. After the meeting, it shows the girl with bruises closed in a dark room, implying that she was trafficked. Our video aims to impact at least 10% of the parents to the proximity of sexual trafficking, making them aware that it is not an unusual crime. Acknowledging this fact is crucial for recognizing the recruitment of a victim and preventing it from happening. This campaign was evaluated by a group of five psychology students and two psychologists. In this group, 83.3% said that the knowledge about sexual trafficking improved and 16.7% said that remained the same. On the overall impact of this campaign, 50% think that was high, 33.3% very high and 16.7% said that was moderated. When asked about the informative content, 66.7% refers very high, 16.7% high and 16.7% moderate. With this campaign, we expect to make the community aware of the reality of this phenomenon, promoting the safe use of social media, keeping in mind that it can be a dangerous place. Although this is our main goal, we also intend to create a protective network that can spread the word and make the Internet safer.

Keywords: sexual trafficking; social media; recruitment; awareness campaign



Analysis and comparison of raw materials and 3Dprinted objects to use in the analysis of 3Dprinted firearms ⁺

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3D printing is an emerging technology in great expansion and has applications in several different areas, such as the automotive industry, aeronautics, footwear, medicine, and prostheses. However, as with many other technological advancements, illicit applications have rapidly emerged, such as producing undetectable and virtually untraceable firearms. This project focuses on determining chemometric profiles of the pre-printed and printed materials by fused filament deposition to discover if it is possible to differentiate between objects printed with filaments of different polymers or the same polymers and different manufacturers. For this work, we selected polymer filaments that have already been used to create operational firearms and that are easily accessible to the public as this increases their probability to be used in the creation of ghost firearms. More specifically, the chosen filaments were PLA, ABS, ASA, PETG and Nylon. The pre-print and postprint materials were analyzed by FTIR (Fourier transform infrared spectroscopy) and are currently being analyzed by Raman spectroscopy, two methodologies suitable for the non-destructive analysis of solid samples. They were chosen not only due to their ease of use but also because they produce easily comparable results and can be quickly applied while on the field to analyze a sample. Analysis of the pre-print filaments and post-print objects shows that it is possible to differentiate between different material, but not between filaments of the same material from different manufacturers. Post-print materials retain spectra like those of pre-print materials, and as such can be used to create a link between samples, with the exception being the Nylon FTIR spectra, which is quite different between the pre-print spectrum and post-print spectrum, however this may have been caused by errors during analysis and storage of the sample. The results obtained show that this methodology can effectively differentiate between different polymers, but not the same polymer from different manufacturers. It can also effectively match post-printed materials to their source pre-print material. However, for this methodology to be applied in future investigations, it will be necessary extend the database of information that is currently available with information about other types of filaments. It should also be extended to 3D printing materials used in other 3D printing methodologies, such as Stereolithography, Selective Laser Sintering, among others. As this is an ongoing investigation, only the results from the FTIR have been obtained, and Raman analysis is still not complete.

Keywords: 3D Printing; Firearms; Forensic Science.



Femicide: Your Silence is the Ultimate Price⁺

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Intimate Partner Violence (IPV) is a complex phenomenon related to social and patriarchal culture regarding the role of women, associated with a value of subordination or inferiority. In contrast, men are related to a value of superiority. Internationally, 45.000 women are killed in the context of IPV, and this crime can be defined as femicide, reinforcing social notions and patriarchal culture. Women are the main victims of this phenomenon, by being killed by their partners or former partners. Thus, women who experience physical, emotional, and sexual violence are prone to be victims of femicide. Firstly, the campaign illustrates a woman as a representation of all who are victims of IPV and at risk of death. The illustration represents the life story of a couple, representing the context of victimization, using the cycle of violence to show typical behaviors that precede/lead to acts of extreme violence. Were also used everyday expressions that refer to cognitive distortions that legitimize violence. The illustrations were gathered into a video to be shared on social media (e.g., Instagram and Facebook). At the end, the victim support numbers were included. The target of this campaign is women, victims of IPV, intending to raise awareness about violent situations and encourage reporting either by the victim or the community. Through this campaign, we intend to inform and raise awareness of the largest number of IPV victims so that they can recognize and report it. One of the images shows the silhouette of a lock to show that violence is often perpetrated inside the home, where we can see a woman being physically abused. This illustration shows the beginning of violence between the couple. Finally, we have an image that references all the thoughts and psychological abuse the woman suffers, expressing the discomfort caused by thoughts of doubt, uncertainty, and fear. This campaign was evaluated (with online survey) by a group of five psychology students and two psychologists. In this group, 57.1% said that the knowledge about femicide improved, 28.7% said that remained the same and 14.3% thinks it's gotten worse. On the overall impact of this campaign, 57.1% think that was high, 28.7% very high and 14.3% said that was moderated. When asked about the informative content, 71.4% refers very high and 28.6% high. As regards the opinion that awareness-raising campaigns contribute to a mitigation of the phenomenon, 42.9% said that contribute a lot, 42.9% said that contribute significantly and 14.2% said that contribute moderately. At the end, 57.2% become more sensitive to this phenomenon, 28.6% moderately and 14.3% said that the campaign had no impact. With this campaign, we aim to draw the attention of women and the community to this phenomenon and its implications. Cultural diversity is increasingly present in our daily lives; the campaigns must consider this factor, so we will translate into English and French to sensitize and raise awareness across borders.

Keywords: femicide; attempted femicide; Intimate partner violence; crime prevention; awareness campaign



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Don't Cut the Beauty! – Female Genital Mutilation⁺

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Female Genital Mutilation (FGM) also known as Female Genital Cutting refers to all procedures involving the total or partial removal of the external female genital organs, or any damage inflicted on them for non-medical or therapeutic reasons, and cultural reasons. This practice is considered a violation of human rights, specifically of children and women, as well as a public health problem that puts the lives of millions of children and women at risk. FGM is essentially based on cultural dimensions as the preservation of virginity, fidelity, and greater fertility of girls and women. FGM brings numerous negative consequences for health at the physical and psychological level, which can manifest themselves in the short, medium, and long term, or even cause death. In Portugal, the prevalence of the practice is intertwined with the immigration factor, verifying that the most significant number of cases occurs with individuals from countries in sub-Saharan Africa and the Middle East. The goal of this campaign is to inform about the practice of FGM and its consequences to the portuguese population. The campaign targets young people (2nd and 3rd cycles of basic education and secondary education, ages between 10 and 17 year old) and teachers (who teach in the cycle off studies as the students). First, the campaign was illustrated through a video that was introduced as if it were a story that will be transposed into reality, in the end it was showed three images used as a metaphor. The first image will have the intention of characterizing the "cut" as a harvest of flowers; in the second a girl places a bandage to symbolize that we can try to heal; and the third represents the scar that is always present. The video will initially address the definition of FGM, as well as the characteristics associated with this practice, followed by a sentence "But this is not just a story" to introduce the statistics and consequences of the same. This campaign was evaluated (with online survey) by a group of five psychology students and two psychologists. In this group, 85.7% said that the knowledge about FGM improved and 14.3% said that remained the same. On the overall impact of this campaign, 42.9% think that was high, 28.6% very high and 28.6% said that was moderated. When asked about the informative content, 71.4% refers very high and 28.6% high. As regards the opinion that awareness-raising campaigns contribute to a mitigation of the phenomenon, 57.2% said that contribute a lot, 28.6% said that contribute significantly and 14.3% said that contribute moderately. At the end, 57.2% become more sensitive to this phenomenon, 28.6% moderately and 14.3% said that the campaign had no impact. With the campaign, we intend to inform about FGM and its consequences so that the target public knows how to identify and knows how to intervene. More aimed at young people, we want them to understand the phenomenon and be able to recognize whether they are victims or potential victims and to identify possible cases.

Keywords: female genital mutilation, female genital cutting, gender violence



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Development of Green Methodologies for Pre-Concentration of Local Anaesthetics in Biological Matrices[†]

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Local anaesthetics like lidocaine, procaine, benzocaine and tetracaine are commonly used in medical and dental treatments. However, they can also be used as cocaine adulterants or as substances of abuse and may present toxicity to the central nervous and cardiovascular systems. In this sense, it is necessary to develop analytical methods that allow, in a rapid and effective way, to monitor these four local anaesthetics in complex biological matrices, while considering the principles of Green Analytical Chemistry. In the present contribution, two green and innovative analytical techniques, namely bar adsorptive microextraction (BAµE) and solid phase microextraction (SPME) with LC Tips, were developed and applied for the preconcentration of the target analytes in biological matrices. The main objective was to compare the effectiveness of the two techniques for the enrichment of the target compounds in aqueous media. The procedures consisted of several analytical steps. First, in the sorption stage, the devices were placed in the target matrix, and by orbital agitation at high speed, the analytes were sorbed in the devices. Afterwards, in the desorption stage, the devices were placed in a suitable solvent which allowed back extraction of the target analytes via orbital agitation or sonication. Finally, the analysis was performed by gas chromatography coupled to mass spectrometry operating in the selected ion monitoring mode acquisition (GC-MS(SIM)). Several sorption parameters such as extraction time, temperature and matrix pH, as well as back-extraction solvent type, time and liquid desorption mechanism, were optimized in aqueous phase using specific design of experiments approach (Box-Behnken Design and Factorial Design). Both BAµE/GC-MS(SIM) and LC Tips/GC-MS(SIM) methodologies appear to be promising analytical alternatives for monitoring these local anaesthetics, given their great simplicity, ease of use and low cost. Under optimized conditions BAµE devices present better efficiencies than LC Tips with recoveries of 46% to 112% for BAµE and 1% to 151% for LC Tips. Preliminary results of BAµE/GC-MS(SIM), using 0,5 mL of urine samples, shown recoveries with similar response as the water samples, indicating low matrix effect. Therefore, this approach BAµE will be selected for validation assays to monitor the selected anaesthetics in biological matrices, starting with urine.

Keywords: local anaesthetics, lidocaine, procaine, benzocaine, tetracaine, BAµE, SPME LC Tips

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Treatment of Anterior Open Bite Associated with Thumb Sucking Habit: A Case Report ⁺

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Anterior open bite malocclusion is characterized by the lack of defined when there is no contact in the anterior dental region between dental arches when the posterior teeth are in occlusion. Anterior open bite impacts oral functions and can influence the quality of life of orthodontic patients. Open bite is a malocclusion with several etiologic factors, such as thumb sucking habit, lingual interposition habit, mouth breathing or due to genetic influence. A detailed diagnosis and anamnesis in patients with anterior open bite is very important to uncover the etiology of the anterior open bite. The aim of this article is to report a case of open bite with a thumb sucking habit treated with a nonsurgical orthodontic treatment. A 17-year-old female patient came to the Egas Moniz University Clinic complaining of anterior open bite. The patient had a bilateral molar and canine class I, posterior crossbite in the left side, lower left second premolar agenesis with the presence of a primary molar and a straight facial profile. Besides, the patient had a severe transverse maxillary deficiency (Pont's Premolar Index: -7,3mm; Pont's Molar Index: -13,1 mm), proinclined upper and lower incisors, and hyperdivergent class I skeletal pattern. The patient had a thumb sucking habit during sleep. Regarding the functional examination, she reported bilateral pain on the Temporalis muscle and TMJ palpation. The patient refused the first treatment plan proposed, combining the orthodontic treatment with an orthognathic surgery, as well as the extraction of the primary molar. Conversely, the patient accepted a non-invasive approach to correct also the posterior discrepancy and its effects. The orthodontic treatment was initiated after obtaining the signed informed consent from the legal guardian of the patient. The orthodontic treatment intruded the molars and corrected the posterior discrepancy with the extraction of the third molars to treat the anterior open bite. Firstly, the orthodontic treatment involved a multibracket mandibular fixed appliance and a bonded acrylic maxillary expander with a tongue grid to intrude molars and correct the thumb sucking habit. Secondly, the upper multibracket fixed appliance was bonded. Upper removable and lower fixed retainers were placed in the end of the treatment. Tongue posture was corrected by miofunctional speech therapy. The treatment duration was 29 months. According to the literature, the open bite malocclusion presents a prevalence of 6% in the permanent dentition and 3% in the mixed dentition. In adult patients, severe anterior open bite cases are often treated with orthognatic surgery. Notwithstanding, orthodontic treatment with molar intrusion and correction of posterior discrepancy appears to be a good alternative with long-term stability founded. In this case report, the anterior open bite was treated with orthodontic camouflage and without any orthognathic surgery. Treatment stability is present with a follow up of 24 months.

Keywords: open bite; posterior crossbite; thumb sucking; orthodontic treatment.



Identification of cocaine and its metabolites in doping control using bar adsorptive microextraction and gas chromatography coupled to mass spectrometry analysis [†]

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Stimulants present in the World Anti-Doping Agency (WADA) list of prohibited methods and substances in sport, is one of the classes with higher percent of positive findings. Cocaine is one of the most consumed stimulants and it's used to enhance the athlete's performance. This drug has a very rapid metabolic disposition, forming two major metabolites, ecgonine methyl ester and benzoylecgonine. Besides these two, the consume of cocaine combined with alcohol forms another metabolite, the cocaethylene. Over the last decade a new sample preparation technique has been introduced, bar adsorptive microextraction (BAµE), as a new environmentally friendly technique. It consists in a small analytical device with an appropriate geometry, coated with suitable sorbent, to retain the target analytes. This approach showed to be suitable for polar and nonpolar substances, reaching high sensitivity, selectivity, and efficiency, with suitable identification limits (1 ng/mL to 10 ng/mL), recoveries between 39.4% and 71.1%, robustness, absence of carryover, as well as stability of the analytes in the final extract, at room temperature, up to 144 hours after extraction. The method still proved to be linear (0.9950 < r^2 < 0.9994) in the tested range and presented good accuracy, repeatability and intermediate precision. The application of the present methodology to samples previously analyzed by the laboratory, showed excellent agreement between the reported and the obtained results. $BA\mu E$ is a greener alternative and a very cost-effective approach to the common sample preparation methodologies, such as liquid-liquid extraction and solid-phase extraction. The present contribution proposes a novel methodology for trace analysis of cocaine and its metabolites in urine samples, using BAµE combined with gas chromatography coupled to mass spectrometry, in compliance with WADA's requirements in doping control.

Keywords: Cocaine; Urine samples; Doping control; BAµE; GC-MS

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Selection of Hydroxypropylcellulose Grade for Paroxetine Formulations 3D-Printable by Fused Deposition Modelling [†]

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Three-dimensional printing (3DP) has recently been attracting the attention of the pharmaceutical community since this technology gives the opportunity to personalize therapy according to patient's needs, re-centering medicines' design on the individual. Fused Deposition Modelling (FDM), the most widely used 3DP technique, involves the prior production by hot-melt extrusion (HME) of a drug-containing polymeric filament, which is then melted and continuously deposited on a surface, layer by layer, building the 3D-printed dosage form. The success of FDM for medicines customization depends on several factors, such as the choice of the adequate polymeric matrix, according to the intended drug release. Recently, cellulose-derived polymers (e.g., hydroxypropylcellulose, HPC) has been extensively studied, in several works, for filament preparation by HME as it can modulate the drug release profile. However, it is crucial to evaluate their properties (alone and in presence of the drug and adjuvants) since these matrix polymers have not been developed specifically for 3DP applications. Based on a comparative study of the drug dissolution profile, this work reports the selection of the most suitable grade of HPC polymer to modulate the release of paroxetine (PRX; used for the treatment of major depression, generalized anxiety and related disorders) from 3D-printed tablets obtained by HME coupled to FDM 3DP. To this end, 3Dprinted tablets were printed from PRX-loaded filaments, previously manufactured by HME, and composed of two different polymeric formulations (HPC LF[™] and HPC GF[™] Pharm). In vitro dissolution of 3D-printed tablets was evaluated, and similarity (f_2) and kinetic parameters, such as $t_{50\%}$ and dissolution rate (DR), were calculated. The dissolution exhibited a profile typically associated with controlled release formulations, particularly useful in the treatment of psychiatric diseases and related to the use of the HPC polymer in both formulations, regardless of its grade. The release profile was superimposable to that of the commercial formulation obtained by a conventional tableting process; the similarity of the dissolution profile between both formulations was supported by a $f_2 >$ 50. On the contrary, the polymeric formulation containing HPC GF presented a slower PRX release profile, as inferred by higher t_{50%} values and lower DR results, when compared to the HPC LF-based polymeric formulation. In this scope, the increase of HPC viscosity associated with the higher molecular weight of HPC GF, impaired the release of the drug. Likewise, the dissolution profile is not comparable with the commercial formulation, despite the closeness of f_2 factor to 50. More than 8h were required for ≥85% of the PRX to be released from 3D-printed tablets composed of HPC GF polymeric formulation (even if it reached 100% at the end of the dissolution test). Overall, this work supports the selection of the HPC LF polymeric matrix as the best option, among those studied, to manufacture 3D-printed PRX tablets by integrated HME-FDM, as a therapeutic strategy in the treatment of psychiatric diseases. 3DP is proven to be capable of mimicking the drug release of commercial formulations with the added value of possible customization to the patient needs.

Keywords: hydroxypropylcellulose (HPC) grade; paroxetine (PRX); printability; 3D-printed tablet; fused deposition modelling (FDM); hot-melt extrusion (HME).

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Analysis of the colour change in composite resins when exposed to colouring agents possible to be found in the oral cavity [†]

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Colour is one of the most important aesthetic parameters in dentistry. All aesthetic restorative materials should simulate the appearance of a natural tooth in terms of colour, and the success of an aesthetic restoration is determined initially by an accurate colour choose and subsequently by its long-term colour stability. The progression of aesthetic expectations and the needs of patients have led to the diffusion of composite resins. This study aims to evaluate the change in the colouration, through the CIE L*a*b* colour system, of nanohybrid and microhybrid composite resins when they are exposed to solutions that can potentially cause pigmentation during a period of 14 days. For 14 days the colouration of 225 composite resin discs (10x2 mm) was evaluated. Three different composite resins were used (Clearfil Majesty™ ES-2 Premium, Elegance Composite Universal and Point 4^{TM}), each divided into 5 experimental groups randomly (n = 15). Each experimental group was applied to 5 different solutions (1 control solution and 4 solutions that potentially could cause pigmentation). The control group corresponded to the solution with artificial saliva. The samples (except for the control group) were incorporated into the respective solutions for a period of five minutes every day for fourteen days. For the remaining twenty-three hours and fifty-five minutes they were kept in artificial saliva. The colouration of the resin discs was evaluated 2 times, before they were applied to the solutions (T0) and after 14 days of application in the solutions (T2), with the spectrophotometer Spectro-Shade[™] Micro. The statistical analysis of the obtained data was performed by using the Statistical Package for the Social Sciences (SPSS) 28.0 software at a p < 0.05. The ANOVA two-way, the Shapiro-Wilk test, and the Levene test were applied. There were significant differences between the composite resins when exposed to the same solution. Similarly, there were significant differences between the solutions in their ability to cause colour changes in the same resin. The effects of exposure to different types of solutions differ according to the type of resin. The Elegance Composite Universal resin showed the highest colour variation for the artificial saliva, coffee, chlorhexidine and energy drink solutions. The Clearfil Majesty™ ES-2 Premium composite resin showed the highest colour variation for the red fruit juice solution. There are highly significant differences between the composite resins when exposed to potentially staining solutions. There are also highly significant differences between the solutions in their ability to cause colour alteration in the same composite resin. The solution corresponding to coffee caused the greatest chromatic variation in all the composite resins studied.

Keywords: Colour Change; Composite Resin; Aesthetics; Pigmentation



Smartphone-based cough data in amyotrophic lateral sclerosis: a potential predictor of functional disability[†]

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Amyotrophic lateral sclerosis (ALS) leads to severe functional disability. Cough depends on both respiratory and bulbar integrity. Correlations between cough sounds and ALS clinical features have been rarely performed. We aimed to assess the relationship between cough (and vocal) sound characteristics with respiratory and bulbar functions in ALS. This was a single-center, cross-sectional study that was part of a broader ALS project (HomeSenseALS - PTDC/MEC-NEU/6855/2020). The study was approved by the local research ethics committee of the Centro Académico de Medicina de Lisboa (CAML-Ref. 146/21) and all participants provided were included after giving their written informed consent. We consecutively collected on-demand cough recordings in ALS patients using a smartphone. The Consensus Auditory- Perceptual Evaluation of Voice (CAPE-V) was performed by a speech therapist. A quantitative acoustic analysis was conducted using time and frequency signal processing on the recordings. Correlation coefficients and multiple linear regression models were used. We recruited 60 ALS patients: 33 females; mean age 60.9; mean 35 months disease duration; 15 with bulbar-onset; and a 34.9 mean ALSFRS-R total. Data from 40 controls were also included. Adjusting to age and gender, our results revealed clear differences between patients and controls on 9 (out of 31) cough sound features. The results revealed main difference between the two groups on sound frequency – significantly lower in ALS (p<0.001 for zero-crossing rate) - likely due to paralysis of laryngeal/bulbar muscles. The distance between signal peaks and sound energy were best correlated to ALSFRS total (p<0.001 and p=0.003, respectively) - demonstrating that worse patients have less intense cough sounds (likely due to respiratory impairment). We are now increasing our controls; and correlating findings with CAPE-V. Our results suggest that cough sound features could emerge as predictors of ALS functional evaluation, at the convenience of using a smartphone.

Keywords: Neurodegenerative Diseases, Amyotrophic Lateral Sclerosis, Personalized Medicine, Digital Health



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Test/retest reliability of the "Community Service Attitudes Scale" for the Portuguese population [†]

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The Community Service Attitudes Scale (CSAS) is a 46-item measure for assessing student's attitudes toward social responsibility in higher education. The scale is based on Schwartz's model of altruistic helping behaviours and is divided into four phases: Activation Step: Perception of a Need to Respond, Obligation Step: Moral Obligation to Respond, Defense Step: Reassessment of potential responses and Response Step: Engage in Helping Behaviour. Each phase has subscales, making a total of eight. Perception of need to respond includes four subscales: Awareness, Actions, Ability, and Connectedness. The Moral Obligation to respond includes two subscales: Norms and Empathy subscales. Reassessment includes Costs, Benefits, and Seriousness subscales. Engage in Helping behaviour includes Intention to engage subscale. The response is based on a seven-point Likert scale ranging from strongly disagree to strongly agree. Previous validation data suggest that the Portuguese measure has strong psychometric properties in terms of validity and internal consistency. The aim of the study was to assess the test-retest reliability of the Portuguese CSAS. Participants were recruited at a higher health school from February to April 2023. They completed an online questionnaire (Google forms) on two occasions, one week apart. Reliability was assessed by calculating intraclass correlation coefficients (ICC). The study was approved by the Egas Moniz Ethics Committee (Process n. 1106 of 30/06/2022) and all students signed the free and informed consent form. Sixty-six university health students (20.4(±0.15) years old, 82% women) participated in both administrations with no missing data. Intraclass correlation coefficients for total scale and each domain were calculated as follows: Total CSAS 0.96, Awareness 0.87, Actions 0.91, Ability 0.93, Connectedness 0.93, Norms 0.94, Empathy 0.86, Costs 0.91, Benefits 0.91, Seriousness 0.92, and Intention to engage 0.90. As a result, the intraclass correlation coefficient obtained between the testretest, by sample size, presented values above 0.90 and low variability which confirms the reliability of the test. The CSAS Portuguese version has excellent test-retest reliability and can be used with confidence by researchers wishing to include it in studies of social responsibility in higher health education.

Keywords: social responsibility; test-retest reliability; portuguese assessment instrument

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Effect of Irrigation with Sodium Hypochlorite on the Bond Strength to Dentin using Different Bonding Protocols [†]

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Pre-endodontic restorations are often performed prior to endodontic treatment and help to create suitable conditions for endodontic treatment, but there is still no consensus on whether to maintain or remove the pre-endodontic restoration for the final rehabilitation of the tooth. The purpose of this study was to evaluate the influence of irrigation with sodium hypochlorite on the microtensile bond strength to dentin with different bonding protocols on pre-endodontic restorations. After endodontic opening of twenty restored human molars, teeth were randomly divided into four experimental groups (n=5): group 1 was not irrigated and the access was restored, while the other groups were irrigated with sodium hypochlorite. Group 2 was restored, group 3 had the endodontic access walls instrumented, and group 4 had CoJet sandblasting and silane application prior to final restoration. Beams (1×1 mm) were obtained from each tooth and submitted to microtensile test in a universal testing machine (1 kN; 1 mm/min). Data were analyzed with one-way ANOVA, followed by Tuckey's HSD (a=0.05). Differences (p<0.05) were observed between groups, groups 3 and 4 showed significantly higher (p<0.05) values of microtensile bond strength than those of group 1. The bond strength after irrigation appears to be higher values when silicatization is performed.

Keywords: pre-endodontic restorations; sodium hypoclorite; bond strength; dentin



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Cardiovagal modulation decreases during exercise regardless of exercise intensity in both males and females [†]

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The regulation of cardiovascular function during exercise is crucial for maintaining homeostasis, with the autonomic nervous system playing a key role. Cardiac baroreflex sensitivity (BRS) and heart rate variability (HRV) are markers of cardiovagal modulation, influenced by age, sex, and fitness levels. Studies suggest sex differences in cardiovagal modulation and blood pressure (BP) during submaximal exercise, with females showing higher HRV and lower systolic blood pressure (SBP) during moderate exercise. However, the mechanisms driving these sex differences and their implications for exercise prescriptions and cardiovascular health are not well understood. This study aimed to compare the response pattern of HRV and BRS indices to acute bouts of cycling exercise at moderate (50% Heart Rate Reserve [HRR]) and vigorous (80% HRR) intensity in active young adult males and females. Based on an estimated medium effect size of 0.27 from the sex differences in HF immediately following exercise, the a priori power analysis suggested that a minimum of 22 participants were necessary (a = 0.05, 1-b = 0.8). To account for possible dropouts, 20 males and 20 females aged 18-31 years were recruited. On the first visit, participants performed two acute bouts of cycling exercise at 50% HRR and at 80% HRR. After a minimum of 48h after the first visit, we also compared the response patterns of indices of HRV and BRS during blood pressure-matched acute bouts of cycling exercise using finger arterial pressure monitoring (Finapres NOVA, NL), with the aim of isolating the effects of differences in SBP. The exercise bouts lasted 2 min after reaching a steady state at each intensity, while the recline bike was standardized for all participants (95°). Time domain and Fast-Fourier-derived frequency domain indices of HRV and BRS were obtained at rest and during exercise and analyzed offline over 2-min time-bins with in-built Matlab software. Changes during exercise in HRV and BRS indices were analyzed with linear mixed models. Exercise decreased cardiovagal modulation in both sexes, although there were no intensity main effects between 50% and 80% HRR, in any HRV and BRS parameter, regardless the sex. Sex-by-intensity interaction effects were observed for root mean square of successive differences between normal heartbeats (RMSSD) (F(3.086)=172.6, p=0.048, ω 2=0.02), high-frequency (HF) (F(7.649)=173.2, p=0.001, ω 2=0.07), and the ratio of low-frequency to high-frequency (LF/HF) (F(5.536)=175.6, p=0.005, ω 2=0.05), which suggests that females had larger reductions in RMSSD (d=32.641 ms; 95% CI: 20.475 to 44.81; p<0.0001) and HF (d=1110.65 ms2; 95% CI: 680 to 1541.78; p<0.0001), when transitioning from rest to exercise at 50% HRR. No differences in the main outcomes were found between visits, which may be explained by the low cardiovagal modulation that females demonstrated during exercise. This suggests that reductions in cardiovagal modulation during aerobic exercise are sex-dependent and occur more during moderate-intensity exercise. These findings underscore the importance of considering sex-specific differences in cardiovagal modulation during exercise, as they may have significant implications for diagnosing, treating, and preventing cardiovascular diseases in females, which have been historically understudied and underdiagnosed.

Keywords: heart rate variability; baroreflex sensitivity; sex differences; autonomic nervous system; blood pressure



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Online education of citizens for health prevention and promotion $^{\rm t}$

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The vast amount of information available today makes it essential to have a reliable source of health information that is easily accessible to communities. By making health information accessible to communities through digital technology, we are contributing to build a community that is more informed about their health and therefore, is more prepared to make informed decisions regarding their own health and engage community action on those matters. To this purpose, the South and Autonomous Regions Branch of the Portuguese Pharmaceutical Society (PPS) has developed a Massive Open Online Course (MOOC) to promote health literacy among citizens on the topic "Safety in Healthcare". This study aims to present the development of three different e-learning courses developed within this MOOC and their results. The development of the courses started in 2019 and were developed by an interprofessional and interdisciplinary team from the PPS and the Portuguese Directorate General of Health (DGS), which defined the topics of the courses and the platform on which the courses are accessible to citizens. Three courses have been created, namely "Safe and rational use of medicines", "Hand hygiene in the prevention of infections" and "Prevention of infections and antimicrobial resistance". On the course "Safe and rational use of medicines" citizens are trained to understand the difference between branded and generic medicines, how to behave responsibly and safely when using medicines, and what to do with expired or unused medicines. The second course focuses on the importance of correct hand hygiene in the prevention and control of infection. "The Preventing Infection and Combating Antimicrobial Resistance" course educated citizens on the correct use of antibiotics to prevent antimicrobial resistance. All courses are available free of charge on a national online learning platform (Platform Project NAU, a national initiative of the Foundation for Science and Technology) and allows participants to work through the e-learning courses at their own pace. Since the launch of the MOOC in June 2019, 28.691 citizens have registered for the three available courses, of which 73.36% (n=21.049) have completed them. The MOOC is achieving great results in terms of both registrations and completion rates, contributing to more and better-informed society.

Keywords: Health Literacy; Online Health Education; PPS



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In vitro efficacy assessment of four decontamination methods in removing Pseudomonas aeruginosa biofilm from dental implants [†]

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Bacterial plaque that accumulates around dental implants leads to peri-implant tissue inflammation and its removal is necessary to preserve the implant. Several strategies have been developed for this purpose. In this in vitro preliminary study, we compared the efficacy of an electrolytic decontaminant (Galvosurge®) with an erythritol jet system (PerioFlow®) and two titanium brushes (R-Brush[™] and i-Brush[™]) in removing 24-h Pseudomonas aeruginosa PAO1 biofilms from implants. P. aeruginosa is a gram-negative bacterium that can be easily cultured under a wide range of conditions and temperatures and has a high ability to adhere to surfaces. For these reasons, P. aeruginosa was chosen as the model bacterium for this research. Changes in the implant surface after each treatment were evaluated by scanning electron microscopy. In the absence of studies comparing defined surface cleaning of contaminated implants, we defined a pilot sample of 4 implants per group, for a total of 20 implants. Titanium SLA implants were immobilised in acrylic models with a tapered defect in the centre and an access hole for implant insertion, exposing the coronal surface. Implants were inoculated with P. aeruginosa and then randomly assigned to each treatment group. A control group was also included, in which implants were equally inoculated but not subjected to any decontamination method. After treatment, decontamination efficacy was assessed by quantifying colony forming units (log10 CFU/cm2) from each implant surface. Except for R-Brush, all treatment strategies were similarly effective in removing *P. aeruginosa* from implants. Major surface changes were only observed on implants treated with titanium brushes. We conclude that electrolytic decontamination, erythritol-chlorhexidine particle jet system and i-Brush[™] brushing have similar performance in removing P. aeruginosa biofilm from dental implants. Titanium brushes caused significant changes to the implant surface, the effects of which need to be further evaluated.

Keywords: Peri-implantitis; implants; decontamination systems; *Pseudomonas aeruginosa*; Electrolytic decontamination; dental implants.



Kinematic analysis comparison between normal and cranial cruciate ligament rupture canine gait analysis: an exploratory study ⁺

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Cranial cruciate ligament (CrCL) rupture is one of the most common causes of dog lameness. The etiopathogenesis of CrCL is not fully known, but regardless of the cause, it results in stifle joint instability and progressive degenerative joint disease. Furthermore, this type of condition causes lameness, cranial tibial translation increased, and internal rotation, and abduction of the tibia. Gait analysis has evolved, it is well-established, and it is a way to perform clinical diagnosis. Subjective analysis has allowed the identification of gait patterns, but results are limited and different agreements between observers can be obtained. Alternatively, objective gait analysis has emerged, allowing clinicians accurately study the canine gait cycle. Specifically, kinematic gait analysis helps effectively and objectively in quantitative evaluation, measuring segments and joints and identifying gait asymmetry. However, the current methods are usually expensive and require extensive data processing, so this study aims an exploratory study which consists of applying a kinematic analysis to characterize and compare a normal canine gait with a canine gait with CrCL with free and opensource software. So, share with veterinaries a simple and useful tool for their daily work. Two dogs (one healthy Labrador Retriever and one Bouvier Bernouis with CrCL rupture) selected by convenience walked ten times with a natural speed in a developed space of two meters near of Egas Moniz Veterinary University Clinic. One high-speed camera collecting at 120 Hz was used doing the bidimensional kinematic analysis. Motion analysis system Kinovea® software was applied to perform data processing. The comparison to spatiotemporal parameters (step time, length, frequency, and speed) and stifle angle between normal and abnormal gait were analyzed with independent samples T-test and Mann-Whitney test. The results indicated that CrCL rupture influenced significatively the canine gait in following parameters, stance phase time (GrGL: 0.56±0.09 s; Normal: 0.46±0.09 s; t=2.576; p=0.019), stance and swing phase in percentage (GrGL: 68.00±0.04 %; Normal: 62.20±0.05 %; t=3.161; p=0.005 and GrGL: 32.00±0.04 %; Normal: 37.90±0.05 %; t=-3.161; p=0.005, respectively), step length (GrGL: 0.73±0.12 m; Normal: 0.92±0.03 m; t=-4.790; p < 0.001), step frequency (GrGL: 1.23±0.14 step/s; Normal: 1.38±0.17 step/s; t=-2.120; p = 0.048) and step speed (GrGL: 0.91±0.22 m/s; Normal: 1.27±0.19 m/s; t=-3.922 ; p<0.001). Regarding the stifle angle, the amplitude did not verify differences between normal and abnormal gaits (stifle angle amplitude: CrCL: 40.4±7.38; normal 41.4±5.91 deg). In sum, CrCL decreased all analyzed parameters, except for the stance and step phase. About the stifle angle signal, when compared with the literature, we verified a similar but inverse signal, and signal differences between dogs, i.e., the gait to CrCL dog started flexion early (around 20%-time gait phase). However, this information should be confirmed with a higher sample and be suggested to veterinary the inclusion of the followup after surgery to ensure that the dog restored all stifle joint range of motion. This study showed that our proposal can be a useful tool for veterinary in their daily routine and allowed us to differentiate the normal and CrCL rupture canine gait obtaining detailed information relevant to clinicians.

Keywords: canine gait; kinematic analysis; cranial cruciate ligament rupture; exploratory study.



ID NOW: an NAAT system solution for rapid and accurate detection of SARS-CoV-2 with VTM sampling [†]

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ID NOW[™] COVID-19 is a rapid molecular test for the detection of SARS-CoV-2. According to the instructions for use, this point-of-care test should be performed on dry nasopharyngeal swab (NPS) specimens. However, this method completely consumes the swab, with the limitation that additional analyses cannot be performed if required. The aim of this work is to evaluate the analytical performance of the ID NOW™ COVID-19 using NPS sampled on viral transport medium (VTM). All NPS were first processed for diagnosis using the gold standard RT-PCR assay (Alinity m SARS-CoV-2, Abbott Molecular) and then tested using the Abbott ID NOW device. A total of 107 NPS were analyze, including 53 samples from COVID-19 community assessment centers and 54 from hospitals (emergency room, urgent care and hospitalized patients). Of the 107 samples, 98 were positive and 9 were negative by RT-PCR. The ID NOW[™] COVID-19 assay detected 84 (85.7%; 95 CI 77.9-91.7) of the 98 positive NPS being the remain 14, false negatives (14.3%; 95% CI 8.8-23.2). None of the 9 negative SARS-CoV-2 samples gave a false positive result with the ID NOW™ COVID-19 assay and no invalid results were found in all samples. When compared to a reference RT-PCR, the overall, positive and negative percent agreement was 87%, 86% and 100%, respectively. The agreement was considered moderate with a Kappa value of 0.502 (50.2%). Due to the number of false negatives, the negative predictive value is 39.1% (9/23; CI: 21.1 - 59.4). However, false negatives were associated with high RT-PCR Ct values. The mean RT-PCR Ct value for discordant samples was 29.6 (95% CI, 28.3-30.9), ranging from 25.4 to 32.9, with a standard deviation of 2.4. The sensitivity of the ID NOW assay for RT-PCR positive samples with Ct values less than 30.0 was 91.8% (78/85 cases). In this study, ID NOW™ COVID-19 assay demonstrate good performance for the detection of SARS-CoV-2 strains compared to the Alinity m SARS-CoV-2 RT-PCR assay, principal for acute infections. The use of NPS sampled on VTM has the great advantage of allowing repeat testing on the same sample without significant loss of sensitivity. However, as for other POC assays, the results of this high-speed assay should be interpreted in a clinical and epidemiological context. In our opinion, POC assays are a promising tool for screening acute medical admissions, urgences, to ensure prompt treatment of patients or minimize nosocomial transmission.

Keywords: Point-of-care; SARS-CoV-2; Rapid diagnostic

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Polyvinyl alcohol/casein hydrogels with oxymatrine for malignant fungating wounds management ⁺

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Malignant fungating wounds (MFW) are a cancer-related complication, caused by infiltration and proliferation of tumour cells in epithelial tissue. MFW are painful, give rise to high levels of exudate, bleeding and malodour. Regrettably, there are cases in which is not possible to close the wound, therefore the only available option is to manage the wound. This can be achieved by conventional dressings, however these do not take into account the specific conditions and needs of these wounds. The ideal wound dressing must provide a physical barrier capable of maintaining the temperature and pH, ensure antimicrobial protection and absorb the exudate, keeping a wet environment in the wound. Furthermore, it must have a low adherence and can't be toxic. The objective of this work was to assess the potential of polyvinyl alcohol/casein (PVA/Cas) hydrogel dressings loaded with Oxymatrine (OXM), an anticancer and anti-inflammatory drug extracted from the plant Sophora Flavescens, for the management of MFW. Casein has several physicochemical characteristics that make it suitable for a hydrogel dressing application, in particular its biodegradability, as well as active biomolecules encapsulation and release potential. Since it is a food-grade biopolymer, it also has benefits such as price, availability and security. Six different hydrogel formulations were prepared following different procedures: freeze-thawing (PVA/Cas-FT); cast drying preceded by crosslinking for 24 and 48 h in the oven at 36°C (PVA/Cas-24CD and PVA/Cas-48CD, respectively). A crosslinker (Genipin, GE) was added to all of them to obtain hydrogels PVA/Cas-FT+GE, PVA/Cas-24CD+GE and PVA/Cas-48CD+GE, respectively. The hydrogels were loaded with oxymatrine by incubation in a solution of drug with 2 mg/mL in NaCl 0.9% for 72 h. Release experiments were carried out in sink conditions using a shaker with agitation (180 rpm) at 34 °C to mimic skin temperature. The best performing formulation was then selected for sterilisation studies, which was done through four different processes: autoclave, microwave irradiation, plasma and high hydrostatic pressure (HHP). Furthermore, the post-sterilization hydrogels were investigated in terms of their physical properties (swelling rate, water content, wettability, morphology, oxygen and ionic permeability), tensile and rheological characteristics, as well as release behaviour. PVA/Cas-24CD+GE showed the best release profile, being able to ensure a sustained release of a significant amount of drug. Regarding the sterilisation method, the hydrogel did not resist to autoclave sterilisation, however the other methods did not compromise the material. More, the HHP sterilisation led to an improvement of the OXM release. The hydrogel properties demonstrated to be suitable for wound dressings production. Overall, the hydrogels proved to be good candidates for oxymatrine loading and MFW wounds management. More work shall be performed to document the drug therapeutic activity.

Keywords: Malignant fungating wounds; polyvinyl alcohol/ hydrogels; Oxymatrine; anticancer, anti-inflammatory; drug release

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Citicoline eluting HEMA-based hydrogels for therapeutic contact lenses ⁺

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Diabetes-related ophthalmological diseases are one of the major causes of morbidity in the world. Citicoline (CIT) is a neuroprotective/neurorestorative drug administered through eye drops to treat ophthalmological neurodegenerative diseases caused by diabetes (e.g., glaucoma, diabetic retinopathy, and anterior ischemic optic neuropathy). Although eye drops remain the primary dosage form for ocular therapy, they provide low drug bioavailability. Soft contact lenses (SCLs), due to their prolonged contact with the eye, have been evaluated in the last decades as alternative drug vehicles for ocular therapy. Nevertheless, their usual low drug uptake prevents the achievement of therapeutic levels. An approach to overcome this issue is to modify the SCLs' material to improve affinity between the matrix and the drug. In this work, hydroxyethyl methacrylate (HEMA) based hydrogels were prepared. Modelling of the drug-monomer interaction was performed using the AutoDock Tools 1.5.7 software (MGL Tools, USA). Since N-(3-Aminopropyl)methacrylamide hydrochloride (APMA), a functional monomer, showed to be suitable to interact with CIT, it was introduced into the composition of the HEMA-based hydrogels, in three different amounts: 100, 200 and 300 mM. CIT (39 mM) was added to the mixture for imprinting purposes. The hydrogels were polymerised using UV radiation, washed extensively to remove the incorporated drug and unreacted monomers, and thereafter loaded with CIT by soaking in the drug solution. In vitro drug release experiments were carried out in sink conditions. The best performing system was sterilised by steam and pressure. A microfluidic cell was used to do drug release tests, mimicking the hydrodynamic conditions found in the eye. Properties relevant for the use of the material in SCLs, e.g., swelling capacity, equilibrium water content, wettability, transmittance, ionic and oxygen permeability, and the refractive index were evaluated. Tensile and rheological tests were also performed. Finally, the samples were analysed by Fourier transform infrared spectroscopy and scanning electron microscopy. The antioxidant activity of the eluted drug was assessed and the adsorption of lachrymal proteins to the hydrogels was studied using a quartz crystal microbalance with dissipation (QCM-D). It was found that presence of APMA favoured both the loading and the release of CIT and that 300 mM APMA led to the best release profile. Molecularly imprinting of CIT in the hydrogels' matrix led to a slight decrease of the initial burst. The drug release kinetics were slower under hydrodynamic conditions, being possible to ensure a controlled release for at least 24 h. More, the hydrogels fulfilled all the requirements to be used in the production of SCLs. In conclusion, the incorporation of APMA as a functional monomer in the HEMA-based hydrogels allowed the optimisation of the loading and release of CIT, presenting a way of improving these materials to obtain therapeutic SCLs.

Keywords: soft contact lenses; citicoline; drug release; functionalised hydrogels; ophthalmological neurodegenerative diseases

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Developing communication skills in higher education - the use of the Pecha Kucha⁺

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Public speaking and public presentation of scientific content are important skills for academics and practitioners. However, failures, inconsistencies, and problems related to time management are frequent during public oral presentations. Not seldom do students find it difficult to synthesise, select and organise information. Given the need to contribute to the development of student's communication skills, a curricular unit (CU) of Information and Communication Methodologies was introduced in the 1st of the study cycle. Among its contents, short communication formats for public speaking are explored, such as Pecha Kucha. The Pecha Kucha presentation model was developed in 2003 by architects Mark Dytham and Astrid Klein with the purpose of making presentations more dynamic and engaging, and these benefits have already been demonstrated. The goals of this activity were to lead students to develop public speaking and communication skills, such as the ability to synthesize and organize information; the structuring and creation of captivating presentations with an eminently visual narrative line; the use of presentation production software. The assessment was carried out through direct observation, giving further feedback on the student's performance. In the academic year 2022-2023, all the first-year students were organized into groups and challenged to prepare a presentation in the Pecha Kucha model, summarizing a scientific article randomly assigned to each group. They then answered a survey in English, made available online, with a set of questions/statements from the study "Do Students Learn Better with Pecha Kucha, an Alternative Presentation Format?". The survey includes 12 statements about the reasons for not including certain elements/information in the presentation (group 1); 17 statements about the reasons for doing so (group 2) and 7 statements aimed at assessing the level of confidence about the presentation in general (group 3). For each statement, students were asked to indicate their level of agreement on a Likert-type scale, ranging from 1 to 5, where 1 corresponds to 'played no role in my decision' and 5 corresponds to 'definitely played a role'. Data were analysed by using descriptive statistical methodologies. Of the 213 students enrolled, 83 (38.9%) participated, by answering an online survey. Data suggest that students did not exclude elements or information in the presentation because of its complexity for the class or the presenters. The main factors for excluding information were related to the issues of the time available for the presentation. The reasons for including information in the presentation were mainly related to the need of contextualisation and the establishment of a narrative line. Students also felt confident answering questions from a varied audience (high school students, dentists, family members, non-teaching staff, teachers, and even the president of the higher education institution). The use of short presentation formats, such as Pecha Kucha, in meaningful and properly planned learning situations, reveals benefits in terms of student motivation, involvement, information synthesis, public speaking and communication skills, innovation, and creativity.

Keywords: communication skills; short presentations; Pecha Kucha; Dentistry.



A VR approach for teaching and learning oral surgical anatomy – preliminary results ⁺

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Teaching, learning, and reviewing human anatomy relies on massive amounts of textual information, accompanied by photos and/or illustrations- typically overloaded with labels, annotations, and captions. Textual descriptions are linear and sequential, which limits the understanding of topographic relationships among anatomical structures and landmarks. Knowledge representations such as graphs have been applied in implantology surgical anatomical education but are not commonly used in teaching practice. Thus, the true pedagogical value of graph representations has yet to be properly evaluated and their potential remains untapped, specifically when considering more contemporary visual mediums such as VR. We aim to address both gaps by evaluating the potential that anatomy maps can bring to teaching and learning topographical anatomy, applied to the domain of oral surgery. Our research questions are: a) Can anatomical maps, displayed inside a VR educational tool, facilitate the learning and perception of anatomical structures and their topological relations for oral surgery? B) Can VR anatomical maps benefit oral surgery education? This work represents a preliminary prototype that was evaluated by 3 dentists experienced in oral surgery that compared two anatomical representations: (i) anatomy maps placed next to a 3D model of a lower jaw with labels vs. (ii) a 3D model of the same lower jaw with the same floating labels. After completion of an informed consent, the following protocol was performed: a) demonstration of the prototype by the examiner; b) free-hand experimentation phase; c) assessment of subjective measurements through questionnaires: i) User Satisfaction Questionnaire (regarding floating graphs and side graphs); ii) System Usability Scale (SUS); iii) NASA-TLX Workload Index; iv) Igroup Presence Questionnaire; d) Semi-structured interviews. Regarding user satisfaction, both side graphs and floating labels were considered useful tools in teaching and learning oral surgical anatomy, although floating labels were preferred over side graphs. Regarding system usability (SUS), a score of 90 was obtained and so, the simulator was considered to have an excellent usability. Perceived workload measured by NASA-TLX guestionnaire indicated very low levels of cognitive workload. Immersive presence questionnaires were "moderate" to "good". Lastly, verbal user feedback obtained through semi-structured interviews, indicated that our approach "eases memorization" and it is "interactive and intuitive", that side graphs and floating graphs "complement each other", that the prototype could be "gamified" by adding a topographical quiz and that it can be increasingly improved to ``plan real surgeries" and "to teach". These results add a significant degree of confidence on future work. The preliminary results were most encouraging in subsequently developing the prototype both for teaching, diagnosis, and oral surgical planning. Further studies are required to assess the real pedagogical value and applicability of our proposed approach with both undergraduate dental students for learning anatomy and implantology postgraduate students for surgical planning.

Keywords: surgical anatomy; virtual reality; anatomical graphs.

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Marginal fit comparison between two zirconia structures ⁺

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The marginal and internal fit of ceramic structures plays a critical role in their success and longevity. Internal fit should be uniform to avoid compromising the strength and/or retention of the restoration, and it should provide an appropriate cementation space. The advancement of dental techniques and materials has enabled the use of oral rehabilitation with enhanced mechanical and aesthetic qualities. CAD/CAM systems have simplified the workflow for dental technicians by eliminating or reducing traditional steps like waxing, layering, and intraoral impressions. The entire process of digitalization, milling parameter calculation, geometric data processing, and the milling process itself are factors that can affect the accuracy of ceramic restoration fit. The quality of the three-dimensional image of a dental preparation directly influences the internal and marginal adaptation of the crowns. The objective of this study is to compare the internal and marginal fit of two zirconia structures manufactured by CAD/CAM. Two frameworks were fabricated using Lava™ (3M OralCare, Seedfeld, Germany) and UPzir® (Upcera, China) to fit a premolar tooth, using Lava™ CAD/CAM and Scan ST (3M[™] OralCare, Germany), Wieland Zeno® 4030 M CAD/CAM (Wieland, Germany) and 3Shape[™] D 200 scan (Wieland, Germany). To simulate cementation, a silicone material with a light consistency, Elite HD + light body (Zhermack SpA, Badia Polesine, Italy), was employed. Two groups were formed based on the ceramic: Lava™ (3M OralCare, Seedfeld, Germany) (G1) and UPzir® (Upcera, China) (G2). To simulate cementation, a standardized continuous pressure of 4.5 kg was applied during 5 minutes according to the silicone manufacturer. The silicone was subsequently removed and divided into four sections for analysis under a Leica® MZ6 magnifying glass (Leica®, Germany). The material thickness was measured at five points: marginal, termination line, axial wall, axial-occlusal angle, and occlusal surface using Image J software (National Institute of Health, USA). Statistical analysis was performed using SPSS[™] Statistics 20.0 software. A two-way Welch ANOVA test was employed with a 95% confidence interval. The greatest mean gap was found in G2 with a 13.67 \pm 8.44 µm, and the lowest in G1 with 8.46 \pm 3.25 µm. There is a statistically significant difference in adaptation between G1 and G2, with G1 demonstrating superior fit (p=0.016). In conclusion, Zirconia Lava[™] appears to have superior internal and marginal fit compared to UPzir®. The material selection for rehabilitation depends not only on the manufacturer but also on the milling and scanning systems used to fabricate the structures accurately. This emphasizes the importance of choosing appropriate systems to ensure successful outcomes in rehabilitation procedures.

Keywords: ceramics; cementation; CAD/CAM



Nitric oxide metabolites and their association with periodontal disease and cardiovascular function [†]

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This study aims to conduct an observational clinical study on the role of the nitrate-nitrite-NO pathway in cardiovascular and periodontal disease. Nitric oxide (NO) is a signaling molecule that plays a key role in several human physiological processes including neurotransmission, homeostasis, cytoprotection, platelet aggregation inhibition, and host defense. The main enzymatic source of NO is the endothelial nitric oxide synthase (eNOS), which regulates the systemic vasodilator tone and blood pressure. The enzymatically produced NO may sometimes be insufficient to maintain its physiological functions, particularly under hypoxic states. In these conditions, alternative pathways for NO production (nitrate - nitrite - nitric oxide pathway) are activated, thus playing an important pathophysiological role in the human body. Therefore, it is important to understand how the complex interplay between nitrate-nitrite-nitric oxide is balanced, particularly, how it is influenced by dietary intake of nitrate and nitrite and by the oral microbiome, which in turn, is affected by oral health. In recent years, this topic has been widely investigated, aiming to understand how oral cavity diseases affect the human N-cycle and what are the repercussions of a dysregulated N-cycle on systemic health. Periodontal disease (PD), for instance, is characterized by dysbiosis of the oral microbiome and has been shown to have effects on the cardiovascular system. On the other hand, several works have shown that salivary nitrate and nitrite levels may correlate with PD and its clinical parameters, such as pocket depth and clinical attachment loss. In this research project, we aim to investigate the periodontal - NO metabolites - vascular homeostasis axis. To this end, we will assess the relationship between PD and the nitrite/nitrate levels in saliva and in blood, while having into consideration the individual diet and oral microbiome. Herein, we will present our latest advances in the development of quick and easy-to-use point-of-care tests (electrochemical biosensors) for both oxyanions that can be used in clinical settings with immediate results. The biosensors are miniaturized screenprinted electrodes that are modified in-house with specific enzymes. The nitrite biosensor, in particular, was first validated using the gold standard method (Griess assay) as a reference. As a main advantage, it requires no sample centrifugation or other pre-treatment as it is not affected by sample turbidity. It is easy to use, quick, and accurate, enabling point-of-care testing for the first time. Currently, we are carrying out a pilot study to assess the salivary nitrate and nitrite levels in patients with and without PD. The results will be correlated with participants' blood pressure to evaluate any possible relationships with cardiovascular diseases. We will also assess the potential influence of dietary intake of nitrate/nitrite-rich food.

Keywords: nitric oxide; nitrite; nitrate; periodontal disease; blood pressure; saliva; oral microbiome; diet; biosensor.

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The Genomic Revolution: The Next Generation Sequencing and the impact on the accurate molecular identification of clinically relevant Gram-negative isolates ⁺

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Next Generation Sequencing (NGS) is a DNA sequencing technology that has revolutionised genomic research. This new technology has enabled rapid identification of bacteria, taxonomy, monitoring, and characterisation of pathogens. Other technologies such as whole genome sequencing (WGS) and metagenomics are also part of this technology. Whole genome sequencing is a method used to determine the sequence of bases in the genome of a given organism, all at once. Conventional methods, such as Sanger sequencing, have played a crucial role in genetic research. Although newer sequencing technologies have emerged, this method remains fundamental for small-scale sequencing projects. Clinically relevant Gram-negative bacteria include a wide variety of microorganisms that can cause a range of human infections. These include wellknown pathogens such as Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumannii. Gram-negative bacteria are also known to be resistant to many antibiotics, making them difficult to treat. This study aims to assess the impact of new genomic sequencing technologies on the identification of clinically relevant Gram-negative strains. A total of one thousand and fifty-five clinical strains of healthcare-associated infections caused by Klebsiella spp. bacteria were studied in this project. These were firstly identified by the microbiology laboratory technologies (Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight Mass Spectrometry: MALDI-TOF MS) and lately molecularly characterized according to DNA sequencing technologies (16S rRNA gene and WGS llumina sequencing). The results obtained using MALDI-TOF have identified one thousand and fifty-five (n=1055) strains as *Klebsiella* spp., of which one thousand and forty-two (n=1042, 98.76%) were identified as K. pneumoniae. The remaining samples were therefore classified as K. oxytoca (n=13, 1.23%). In contrast, the following species



and subspecies were identified from the molecular approach: *K. pneumoniae* (n=978, 93.86%); *K. pneumoniae subsp. rhinoscleromatis* (n=21, 2.02%); *K. pneumoniae subsp. ozaenae* (n=19, 1.82%); *K. quasipneumoniae* (n=4, 0.38%); *K. quasipneumoniae subs. quasipneumoniae* (n=1, 0.10%); *K. quasipneumoniae subsp. similipneumoniae* (n=5, 0.48%); *K. variicola* (n=5, 0.48%). For *K. oxytoca*, the following subspecies were misidentified: *K. oxytoca* (n=9, 69.23%); *K. grimontii* (n=2, 15.38%); *K. michiganensis* (n=2, 15.38%). In conclusion, the molecular identification provides an accurate identification of bacterial species. Compared to classical techniques, molecular methods can provide advanced knowledge about the *K. pneumoniae* and *K. oxytoca* complex species. Therefore, the results showed that fifty-nine strains (5.59%) were misidentified using traditional methods. Finally, it is important to promote further studies on their clinical impact, such as antimicrobial resistance, virulence, and disease severity, as this is still unclear.

Keywords: Next-Generation Sequencing; Conventional methods; *Klebsiella pneumoniae*; *Klebsiella oxytoca*; Antimicrobial resistance



Raman spectroscopy applied to blue and green ink discrimination – A pilot study [†]

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The analysis of the ink of common writing instruments is one of the most relevant areas of modern criminalistics in document fraud since the specific characteristics of inks can answer identification, comparison, and/or dating questions in requested forensic examinations. This work aims to contribute to the discrimination of blue and green inks from common handwriting instruments through a nondestructive technique where the full Raman spectra analysis with Knime software is explored in order to build a predictive model. Eleven pens (5 blue and 6 green) were selected, from 26 blue and green pens, after signal Raman screening. These inks were deposited in notebooks, created with current paper (Navigator) in circular areas with different stroke overlaps in each quadrant, to optimize the acquisition conditions. Raman spectra of the ink circular area deposits were acquired using a Raman Mira DS equipped with a laser with an excitation wavelength of 785 nm (100mW), and a spectral range of 200-2300 nm. The equipment provides a spectral resolution of 8-10 cm-1 and a measuring spot of 0.042-2.5mm. The detection technique used is Orbital Raster Scan (ORS), where a tightly focused beam (0.04mm) scans a relatively larger area compared to the conventional Raman, averaging the information. It has a universal fixture with three positions that allow the surface of objects to be analyzed at three different distances: in contact and at 3mm and 5mm. After acquiring 3 replicates of the Raman spectra for the pen inks, the respective baseline was adjusted using Spectragryph software version 1.2.15. The baseline of the spectra was adjusted 2 times. The Raman Spectra files of each ink were split into 2 databases to create a predictive model with KNIME Analytics Platform software version 4.6.0: a) training database (which contained the data from 2 replicates for each of the dyes, the averages of the triplicates and the average between each two replicates) and the b) validation database (which contained 1 of the replicates that were not present in the training database). Based on the Raman spectra obtained, a predictive model was built using KNIME software, with a discriminatory power of 72.7% was obtained. This study allowed the construction of a potential tool for discriminating pen inks through non-destructive techniques that could in the future be used in a real-world context.

Keywords: Questioned document, Inks, Raman spectroscopy, Handwriting Instruments.

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Facilitating WES screening for genetic disorders using AI-driven bioinformatics ⁺

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Whole Exome Sequencing (WES) using Next Generation Sequencing (NGS) technologies is a clinically accepted diagnostic strategy for identifying pathogenic genetic variants in children and adults to determine the cause of genetic disease. Databases have accumulated over time containing information on an ever-increasing number of pathogenic variants, leading to more than eight thousand gene-disease associations. Public and private healthcare facilities are beginning to use this data as a front-line tool, over conventional techniques, to diagnose paediatric rare genetic diseases. However, analysis using bioinformatics tools can be very complex and requires specialist skills and training, hence it can take several weeks from sample to diagnosis. The technique's relative complexity and high labour intensity (and therefore cost) has been a limiting factor for its application in the general population as a screening tool to prevent rare genetic disease. Artificial Intelligence (AI) is considered a solution for automating complex analysis and decision-making. In this work, we present 2 case studies where we applied an AI-driven bioinformatics framework in a diagnostic and a preventive scenario, respectively. Firstly, to identify a suitable gene variant candidate in an infant with a suspected genetic disease. The AI identified 15 suitable candidates from ~110,000 gene variants. Furthermore, the top-ranked variant was the one chosen by independent molecular geneticists as causative of the phenotype. Secondly, we applied AI to screen for all reported genedisease associations in a phenotypically healthy couple at the preconception stage to predict the risk of having a child with a genetic disorder. The AI analysis was substantially faster (a matter of seconds) than using conventional bioinformatics tools. Our results support the concept that AI-driven bioinformatics is a scalable solution for rendering accurate results and enabling a more widely available democratized genetic screening for rare diseases.

Keywords: Genomics; Bioinformatics; Rare Diseases; Artificial Intelligence; Pre-conception

Acknowledgments: We acknowledge Molmart Ltd for supplying the WES data of the 2 clinical cases and for the use of the necessary software to perform the analysis.



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The effect of substrate colour on lithium disilicate ceramic with two thicknesses ⁺

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Dental aesthetics, including the shape, texture, alignment, colour, and size of the anterior teeth, as well as facial and oral symmetry, play a crucial role in achieving an aesthetically pleasing smile and contributing to patient satisfaction. The importance of aesthetics has significantly increased in modern society, with patients and dentists alike placing greater emphasis on appearance. As a result, the ability to perform aesthetic treatments has become a crucial factor in a professional's reputation and the success of their clinical practice. To achieve total success in an aesthetic case, it is essential to precisely comprehend and evaluate the substrate colour of the teeth. This factor has a significant impact on the selection of ceramic type, thickness, and cements. This study aims to evaluate in vitro the colour changes of two substrates when lithium disilicate ceramics with two thicknesses are employed as a restorative material. Forty IPS e.max® CAD (HT) A1 ceramic discs (Ivoclar Vivadent, Schaan, Liechtenstein) were fabricated with thicknesses of 0.5mm and 0.8mm. Forty Filtek™ Supreme XTE Universal Restorative Body (3M ESPE, Minnesota, USA) composite resin samples, A2 and A3 shades, were fabricated with a 1mm thickness. The samples underwent a 15-second polishing process using a LaboPol-4 polisher, utilizing a sequence of sandpapers with varying grit sizes, at a rotational speed of 100 rotations per minute. The composite resin's colour was measured using a Spectro Shade[™] spectrophotometer. After matching the composite resin samples with the ceramic ones, the colour was measured again. Colour change analysis was performed using the ΔE value in the CIELab system. Statistical analysis was conducted using SPSS[™] Statistics 20.0 software, employing a two-way ANOVA test with Bonferroni's method for multiple comparisons and a 95% confidence interval. The greatest colour change was found when using a 0.8mm ceramic on an A3 composite resin base (11.05 ± 0.30) , while the smallest change was observed when pairing an A2 base with a 0.5mm thick ceramic (7.95±0.31). A greater ceramic thickness results in a higher colour change, showing statistical significance (p < 0.05). When we turn the substrate from an A2 to an A3, regardless of the ceramic thickness used, the ΔE value increases significantly (p<0.001). In conclusion, using different disilicate ceramic thicknesses can take an important role when the clinical approach is to mask the substrate. The substrate colour is a crucial factor to consider during dental preparation, as it directly influences the required thickness for achieving aesthetic success with the ceramic material.

Keywords: ceramics; dental veneers; spectrophotometry; dental materials

Acknowledgments: Thanks to 3M ESPE for the sponsorship and supply of Filtek[™] Supreme XTE Universal Restorative Body.



Predicting Cancer Prognostics from Tumour Transcriptomics using an Auto Machine Learning Approach ⁺

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Cancer is a worldwide flagellum, leading to millions of deaths per year. Cancer treatment efficacy often depends on the treatment choice, which depends on the genetic factors and tumour heterogenicity. Cancer prognostic biomarkers are a way to access treatment responses and are considered a promising precision medicine approach for helping decision-making during cancer treatment. However, currently used cancer prognostic biomarkers still have low predictive power. Tumour transcriptomics is an affordable and accurate precision medicine approach enabling access to numerous biomarkers. Applying machine learning (ML) frameworks on transcriptomics data can potentially find biomarker signatures for binary classification (yes/no) of cancer survival with predictive power. We tested the potential of ML for generating transcriptomics-based cancer prognostic predictors. An auto ML framework (TPOT) was used to systematically test thousands of algorithms on lung, breast, and kidney cancer transcriptomics datasets. The model's performance was evaluated, and their optimal sensitivity, specificity, accuracy, and computed ROC-AUC were reported. Breast and Kidney cancer prognostic models performed with good sensitivity (94%) but poor specificity (<66%), whereas the Lung cancer prognostic models had reasonable specificity (>83%) but poor sensitivity (59%). Kidney cancer prognostic models showed good predictive power with a ROC-AUC and accuracy >70%. The results support the potential for applying auto ML approaches for the future development of cancer prognostics tools based on transcriptomics data.

Keywords: Bioinformatics; Cancer prognostics; Machine learning; Transcriptomics



Tooth Wear progression in enamel and *Lava*[™] Ultimate – in vitro study [†]

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Tooth Wear is becoming increasingly relevant due to the increase in life expectancy and the decrease in edentulism. The loss of hard tissue resulting from this problem is irreversible, highlighting the need to be aware of its progression and make an early diagnosis. The aims of the present in vitro study were to evaluate and compare Tooth Wear by attrition in enamel and Lava[™] Ultimate specimens, as well as evaluate the ability of the CEREC Primescan® intraoral scanner to measure Tooth Wear progression. The study conducted contains a control group consisting of 12 pairs of specimens, flat enamel (E) and cuspid (CE), and an experimental group consisting of 12 pairs of specimens, Lava[™] Ultimate (C) and cuspid (CC). These were submitted to the attrition protocol in the Chewing Simulator, consisting in three wear cycles (24h, 48h and 96h), in artificial saliva. For each sample, an initial scan was made before starting the attrition protocol, and after each cycle. The volume loss of the samples (%, vol rel) was calculated and statistically analysed. As a result, there was a reduction in volume over time, which was statistically significant (p<0.0005) for all groups. Analysis of wear between groups showed significant differences between the CC and E groups at t_1 (p=0.026) and t_2 (p=0.039), between CC and C at t_2 (p=0.038) and between CE and E at t_4 (p=0.037). In conclusion, CEREC Primescan[®] can measure Tooth Wear over time. Point differences were found between enamel and ceramic wear. CEREC Primescan® has proven to be a potential clinical tool to assess, diagnose and monitor Tooth Wear.

Keywords: Tooth Wear, Tooth Wear Quantification, Attrition, Intraoral Scanner

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ChatGPT a promising learning tool? An exploratory conversation on food labels ⁺

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ChatGPT, a generative pre-trained transformer chatbot developed by OpenAI, was launched in november 30th, 2022. This large language model based on Artificial Intelligence (AI) can understand and generate human-like responses using a text-based interface. In six months only, ChatGPT has received great attention by the medical and health sciences communities, including nutrition and pharmaceutical. One of the potential values of this chatbot is to assist students in their education, as the free application is easily accessible to the general public. However, the limitations of this AIbased tool are guite unknown. The information of the food label is crucial for consumers, since it could be used for their choices. It is also a valuable tool from the Public Health point of view, to monitor-preferences and consumption and to promote healthy lifestyles and nutrition worldwide. Here we aimed to evaluate the accuracy and the completeness of ChatGPT in answering questions regarding knowledge and skills on food labelling regulatory compliance, Nutri-scores validation, nutritional food decoder use and nutritional food recommendations for infants and young children, aged between 0 and 36 months; ChatGPT generated answers to 13 questions regarding the themes mentioned above were evaluated by a student and their supervisors (S1 and S2 with greater expertise in food law and public health, respectively) graded for accuracy and completeness (5 point ratting scale: 1-not at all; 2-slightly; 3-moderately; 4-very; 5-extremely and complete with additional information) and the scores summarized by descriptive statistics; The student accuracy median score and the interquartile range (IQR) for all ChatGPT answers reviews were 4 (4-5), respectively, and the corresponding supervisors values were 3 (2-5) (S1) and 4 (3-5) (S2); the student median completeness score and IQR values were similar to the accuracy ones 4 (3-5)) and their supervisors values were 3 (2-4) (S1) and 4 (3-4) (S2). The food Portuguese law answers (6/13, 46%) had the lowest median scores, ranging 3 to 4 (IQR 2-3 to 3-4; min. 1- max. 5) for accuracy and 2 to 3 (IQR 1-2 to 3-4; min. 1- max. 5) for completeness. The overall accuracy evaluation was good (median - 4; IQR 3-5) but the completeness was more a fair (median - 3; IQR 3-4) as ChatGPT answers don't include references. These results showed that the free version of ChatGPT has limitations accessing information in Portuguese and from images; and our study suggests that ChatGPT is a useful tool for guidance and interpretation of food labels. This chatbot seems to be very interesting in learning, especially in an initial phase, if the information provided is verified. However, it still needs to be improved because it has a wide range of topics that are important for different science and fields and research applications. Moreover, it is practical and friendly for application in Public Health studies and education.

Keywords: food labelling; ChatGTP; regulatory compliance; Nutri-score; nutrition food decoder; infants and young children, food recommendations



P5 Food and nutrition security from sustainable food systems, while preventing and mitigating pollution



Chemical and Microbiological Risks in Insectbased Food Production ⁺

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The use of insects as an alternative source of protein for animal and human consumption is a viable option for meeting the challenge of providing food for a growing population. Additionally, the circular economy offers a potential solution to the challenges of food production, with positive impacts on both the economy and the environment. However, concerns have been raised regarding the safety of insect-based products due to the potential presence of chemical and microbiological contaminants. In fact, the use of insects in feed and in food production requires a systematic and more in-depth studies due to the complex technological processes involved. To address this issue, this study was conducted to assess the chemical and microbiological risks associated with insect meals and in fish feed throughout the entire food production chain. The study focused on the use of agro-industrial by-products to feed two insect species (Tenebrio molitor and Hermetia ilucens), and the subsequent use of insect protein as feed for aquaculture fish. Conventional microbiological methods and ELISA were used to detect pathogenic microorganisms and mycotoxins, respectively, while wavelength dispersive X-ray fluorescence spectrometry (WDXRF) was used to screen for elements of toxicological concern, such as heavy metals (Hq, Cd, Pb), and As. The study found that the processed insect larvae and fish feed formulations tested were free of toxic and potentially pathogenic microorganisms as well as toxic elements (Hg, Cd, Pb and As), suggesting that agro-industry by-products can serve as an environmentally and economically viable source of protein for both animal and human consumption. In conclusion, by using agro-industrial by-products to feed insects, we can ensure a sustainable source of protein for both animal and human consumption while taking steps to promote a better economic and environmental sustainability. It should be noted that although the results obtained are promising further in-depth studies, including a broader study of other chemical contaminants not included in this work, are needed to ensure the safety of insect-based products in food and in feed production. Nevertheless, these preliminary results offer hope for the use of insects in the circular economy to promote sustainability in the food chain.

Keywords: Circular economy; agroindustry by-products; fish feed production; *Tenebrio molitor*; *Hermetia ilucens*; microbiological risks; chemical risks.

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P6 Maintaining an innovative, sustainable, and globally competitive health-related industry



Impact of acid rain with different pH values in Monstera deliciosa Plants⁺

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The present research experimentally outlines the reaction of Monstera deliciosa plants to the effects of acid rain, at various pH levels, and whether these changes affect differently this type of plant. The experiment was carried out in a period of 19 days, where temperature was recorded every day, ensuring this variable would not affect the obtained results. Sulfuric acid was used to simulate acid rain, and the prepared solutions had 4 different pH values: 2, 3, 4 and 5. In order to analyse the effect of the different acidic solutions, 15 M. deliciosa plants were tested, and 3 trials were undertaken for each pH; 3 of the 15 plants were intentionally left without water for a week before the start of the experience, in order to simulate dead or a poorly-maintained plant. Solutions of 250 mL with sulfuric acid and distilled water were prepared to achieve the desired values of pH through dilution. For the first half of the experiment, the plants were given 10 mL of the respective solution 3 days per week. After 10 days, the volume of the solution was doubled to 20 mL, resembling an increase in acid rain directly falling on the plant's soil. Paper chromatography was performed to analyse the deterioration of chlorophyll. A leaf of each plant was taken, being placed in its respective and identified mortar, where 5 mL of di-ethylether were added, crushing the leaves with the solvent using a pestle. Chromatography paper was cut, leaving a 1 cm margin at the bottom to be dipped in hexane. Spectrophotometry was carried out to assess how well the plants absorb radiation while being treated with sulfuric acid solutions of various pH levels. The values read by spectrophotometer were obtained by the wave lengths 330 nm to 520 nm. Using a mortar and pestle to crush the leaves with distilled water, the liquid was then pipetted onto a funnel with filter paper to catch any contaminants and drain the clean extraction of the leaves. A cuvette containing this extraction was then put into the spectrophotometer. Paper chromatography was performed four times, once in the beginning, twice in the middle and once in the end of the experiment. Spectrophotometry was performed twice, once in the middle and once at the end of the experiment. Paper chromatography showed that the plant's pigment began splitting and the chlorophyll got furtherly deteriorated. On the eighteenth day of experimentation, chlorophyll was nearly completely degraded. The pouring of the sulfuric acid mixture affected the absorbance values measured by spectrophotometry with a oneweek interval. Using quantitative data, it was possible to determine that plants with the lowest pH levels of acid lost their capacity to absorb light and radiation more quickly, since their absorbance values were lower and so it is possible to conclude that acid rains affect Monstera plants, which is correlated with the decrease in absorbance values at 430 nm, the maximum absorption of chlorophyll.

Keywords: Acid Rain; Monstera deliciosa; Sulfuric Acid; pH; Paper Chromatography; Chlorophyll; Spectrophotometry.



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Efficiency of antacids regarding its components ⁺

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This research investigates the possibility of creating an antacid solution using organic compounds found in everyday homes, with the aim of achieving an efficiency level comparable to commercial antacids. The study compares solutions containing three organic compounds (garlic, ginger, and neutral gelatine) with one of three different chemical components found in commercial antacids (sodium alginate, sodium bicarbonate and calcium carbonate). The efficiency of these solutions is evaluated through a comparative analysis using a titration with hydrochloric acid as the titrant. The objective is to determine if a solution based on organic compounds can effectively neutralize stomach acidity and reach a level of efficiency like commercial antacids. The background information provides an overview of antacids, the role of gastric acid in the stomach, and the composition and function of the chemical components in commercial antacids. The methodology begins on determining the adequate amount and concentration of the titrant and then to create the other solutions. After some calculations, the analysed amount of each solution is determined and created and, finally, each variable is analysed through a titration process, finding its equilibrium point. The results demonstrated that, from the four solutions, the most effective was the pharmaceutical antacid, with an average of HCl volume used of 15.98 mL to neutralise the solution. Nevertheless, from the other three solutions, the solution with sodium bicarbonate and the three organic compounds was also very effective, having an average of HCl volume used of 13.75 mL. The solution with calcium carbonate did not need much HCl to neutralise the solution, having a mean of 0.65 mL and, finally, the solution with sodium alginate did not work, since it instantly jellified, not being able to be analysed. At the end, it was concluded that, although none of the solutions were more efficient than the pharmaceutical antacid (15.98 mL), the one with sodium bicarbonate (13.75 mL) was not so far from the control variable, when compared to the other two solutions, which demonstrated a low or null efficiency (average of 0.65 mL and 0 mL of HCl used).

Keywords: solution; antacid; indigestion; efficiency; organic compounds; green chemistry.



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The effect of phosphorous on the sweetness of strawberries ⁺

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Strawberries are fruits very appreciated and worldwide consumed. Their sweetness is an appealing quality to consumers and thus needs to be taken into consideration when growing this plant. In this work the correlation between the Phosphorous content supplied to the plant and the carbohydrates found in strawberries was studied, in order to determine the importance of this element and its optimal concentration. When phosphate is bound to sugars, it supplies some of the energy needed to generate various sugars, carbs, and starches, which are then converted into all of the plant's organic compounds. The majority of carbohydrate transformations in plant cells involve phosphorylated forms, thus, it was predicted that a larger concentration of the element would influence the presence of carbohydrates in the fruit. Different concentrations of phosphorus (30 mg/L, 37.5 mg/L, 45 mg/L, 60 mg/L) were provided to strawberry plants (*Fragaria* x ananassa Duch.) with six plants per group, via plant solution before, during, and after pollination (for a duration of 5 weeks). All the subjects equally separated and receiving the same luminosity. Upon collection, strawberries were stored in freezing temperatures. Results were measured with a portable refractometer in ^oBrix. Brix refers to the percent carbohydrates in 100 grams of juice. Carbohydrates in strawberries consist mainly of sucrose, glucose, and fructose, however, for the flavor of the fruit, the individual concentrations of the different sugars are less important than the total sugar concentration. The concentration of phosphorous provided to strawberry plants showed to have a strong positive correlation with the sugar content found in the fruit (r^2 =0.965), with an average ^oBrix reading of 10.93 for a 30mg/L phosphorous content, 11.29 ± 1.54 for 37.5 mg/L, 11.94 ± 2.97 for 45 mg/L and 12.46 ± 2.80 for 60 mg/L.

Keywords: Strawberry plantation, phosphorous, carbohydrate content



Evaluation of Vegetable By-Products as Feed Bases for Black Soldier Fly Larvae ⁺

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The Hermetia illucens, commonly known as the Black Soldier Fly (BSF), is gaining recognition in animal production for its significant potential in solving the food agri-industry by-products problem and contributing to the production of alternative protein and energy sources to animal feed through circular economy. This insect species offers numerous advantages, including excellent capabilities in bio-digestion and bio-conversion into organic fertilisers and high-value protein biomass, using agroindustrial by-products in an efficient and profitable manner. Therefore, in the last decades, much research has been directed towards the influence of different food by-products on the growth and performance of BSF larvae. In view of this, a study was performed in the scope of ADVAGROMED project to evaluate and select vegetable by-products with greater availability and within a radius of 15 km of Ingredient Odyssey location in Santarém, Portugal, in the period from September to December and to assess them as feed basis for BSF larvae. The selected vegetable by-products were olive pomace and melon by-products, obtained from agro-industries in the region and formulated to meet the needs of the larvae. The experiment consisted of three treatments (olive pomace based, melon based, olive pomace + melon based) and a control group (cereal-based diet). All the 3 treatments were formulated for the same crude protein as the control group. The juvenile larvae were inoculated directly into the diets and the bioconversion process was performed in an environmentally controlled room at a temperature and humidity suitable for their development. The bioconversion process was monitored daily until the substrate was fully biodigested. At the end the content was sieved to separate the bioconverted substrate (organic fertilizer, Frass) from the larvae. The collected material was weighed and the parameters evaluated were the total frass production, the total weight of the larvae after biodigestion and the average unit weight of the larvae, per experimental unit. The results showed that the larvae performed well in all groups and that the ones, fed on melon showed a higher weight compared to the other treatments. The results showed that the melon by-product is an interesting alternative for larval feeding, probably related with the sugar content of the diet. The use of BSF larvae for the valorization of the by-products into valuable biological biomass is of of great importance.

Keywords: Black soldier fly, By-products, fertiliser



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Characterization of frass prepared with different types of manure ⁺

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In recent years, the Black Soldier Fly - (BSF) (Hermetia illucens) has been studied due to its effectiveness in the bioconversion of organic matter from different sources as agro-industrial byproducts such as vegetables, fruits and other plant materials to produce larvae biomass that can be used as raw materials for feed. Nevertheless, this great capacity of biodegradation can also be used for residues bioconversion namely livestock effluents, which are a huge environment challenge nowadays. These types of residues can be converted, and the excrement is obtained at the end of this bioconversion process. According to Regulation (EU) No 142/2011, 'frass' should be defined as a mixture of excrement from farmed insects with parts of dead insects, dead eggs and food substrate. This animal by-product of the BSF larval cycle (BSF frass, BSFF) is very rich in nutrients and minerals and therefore has been used as an organic fertilizer for all types of crops. The centesimal according to AOAC methods and mineral composition was determine by atomic absorption spectrometry. The BSFF has been studied and investigated in terms of the different bioconversions carried out in the BSF due to the composition of the organic matter. Therefore, the main focus of this study was to analyze the composition of the BSFF with different treatments: chicken manure (CH), bovine manure (BO) and swine manure (SW) manure and the centesimal analyzes were dry matter (DM), total moisture (TM) and crude protein (CP) and the minerals analyzed were nitrogen (N), phosphorus (P), and potassium (K). The experiment took place in the EntoGreen/I&D in research and development, located in Santarém, Portugal in 2021. The comparison between treatments was performed using the SPSS software (version 27) and the comparative analysis between groups was performed using the Mann-Whitney test with a=5 %. According to our research the DM, TM, parameters, there was no significant difference between treatments a < 0.05. The CP was higher in BO and SW an average of 16.02 ±0.87. Regarding the amount of nitrogen, the SW and BO treatments presented considerable values $16.48 \pm 0.31\%$ and $15.56 \pm 0.56\%$ respectively, when compared to the treatment CH. In P concentration there was no significant difference (a=0.05) between CH and BO. In the comparison for K, the SW treatment obtained the highest concentration $3.11 \pm 0.10\%$. Among the analyzes of the centesimal composition of the frass, all treatments obtained considerable total humidity within the indicative values of the literature. The lower the humidity, produced less volatile fatty acids and phenols, resulting in less odorous excrement. The results of the minerals N, P, K are essential for the nutrition of the plants. Among the treatments the most indicated BSFF would be the SW and BO treatment, which resulted in higher concentrations of these minerals. However, a more complete analysis of the minerals would be indicated for a future study.

Keywords: BSF, organic fertilizer, Hermetia illucens



Next-generation Shotgun Proteomics of Marinederived Actinomycetes ⁺

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Actinomycetes Streptomyces aculeolatus belong to the MAR4 streptomycete lineage and are known for producing secondary metabolites, mainly hybrid isoprenoids (HI). These compounds have great potential as antibacterial, antifungal, and anticancer agents, which makes them attractive to the pharmaceutical industry. In previous works, our group collected a total of 400 actinomycetes isolated from marine sediments in the Madeira Archipelago (Portugal). Six of these microorganisms belong to the S. aculeolatus species, as determined by a phylogenetic analysis based on the sequencing of the 16S rRNA gene, all producing HI with distinct bioactivities, except one, which can serve as a negative control of the secondary metabolism. In this context, we performed a preliminary differential proteomic analysis based on two-dimensional electrophoresis (2DE) of the six strains. From this comparative study, we selected the four strains with a distinct proteome/bioactivity profile for further characterization through an exhaustive shotgun nanoLC-MS/MS analysis. To this end, we performed four independent growths of each strain in a starch, yeast extract, peptone, and seawaterbased medium. Cells were harvested after 4 and 7 days of growth, the proteins were precipitated with trichloroacetic acid, in the presence of β -mercaptoethanol. After a washing step with acetone, the samples were resuspended in Laemmli buffer and heated, prior to a short SDS-PAGE separation step. The proteins were then proteolyzed with trypsin. Finally, the tryptic peptides obtained from each of the 32 samples (four biological replicates and two different growth times: 4 and 7 days) were analysed by tandem mass spectrometry with a Q-Exactive HF Orbitrap mass spectrometer coupled to an UltiMate 3000 LC system with 90 min gradient. A total of 1,733,241 MS/MS spectra was delivered. From these, a total of 766,878 Peptide-to-Spectrum Matches (PSMs) were interpreted, resulting in a ratio of 44% assigned MS/MS spectra. The datasets were normalized giving a confident identification of 31,024 unique peptides and 2492 proteins. A PatternLab differential analysis of the same strains comparing the data for the two growth conditions was performed considering a T-fold and the associated p-value cut-offs at 1.5 and 0.05, respectively. The statistical analysis showed that the proteomic datasets for the 32 samples were distinguished by the PCA test, and no biological replicates was considered as an outlier. To compare data between these strains, all proteins common to the negative control strain proteome were eliminated and the Venn diagrams were plotted. In future work, we will intend to perform the network construction using the STRING database to find potential metabolic pathways involved in the production of bioactive secondary metabolites produced by the *S. aculeolatus* strains under study.

Keywords: Marine actinomycetes, bioactive compounds, secondary metabolism, proteomics, nanoLC-MS/MS

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Influence of cavity wall thickness on the color adjustment potential of single-shade resin composites [†]

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Single shade resin composites have been introduced to the market to simplify shade selection as these materials tend to mimic tooth colour through a physical mechanism (structural colour). Compared to multi shaded resin composites, these materials have a greater ability to adjust their color to the surrounding structures, thus offering a greater colour adjustment potential (CAP), which can compensate for any mismatch and contribute to the aesthetics of the restoration. As single shade composites rely on the surrounding structure to express colour, it could be hypothesized that this ability could be influenced by the thickness of the surrounding tooth structure. The aim of this study was to evaluate the influence of cavity wall thickness on the CAP of single shade composites. Single (S) and Dual (D) specimens (\emptyset = 11 mm; 4 mm thick) were fabricated. The S specimens were made with all the evaluated composites (Vittra APS DA3 -V, Vittra Unique - VU and Omnichroma - OM). The D specimens were made with the control composite (V) to simulate the dental structure, but they had cavity preparations (\emptyset = 5 mm; 1.5 mm depth) in different positions (leaving a wall with a thickness of 3, 2 or 1 mm), which were restored with one of the test composites (VU and OM). The L*a*b* coordinates were obtained from digital photographs and the measurements were taken at 3 points in the D specimens (P0 - restoration centre; P1- restoration periphery; P2 - control composite) and 1 point in the S specimens (P0 - central area). The ΔE_{00} data were calculated using the CIEDE2000 colour difference, considering the following measures: (1) P0-P1 - difference in test composite (ΔE_{00R}); (2) P0 - P2 – difference between test (central) and control composite (ΔE_{002C}); (3) P1 - P2 – difference between test (peripheral) and control composite (ΔE_{002P}). CAP was calculated using ΔE_{001} (colour differences for test composite, between D and S specimens) and ΔE_{002C} and ΔE_{002P} for central (CAP_{002C}) and peripheral (CAP_{002P}) areas, respectively. Data were statistically analyzed using two-way ANOVA, Holm-Sidak, and paired t-tests (a=5%). Colour differences were also interpreted considering the 50:50% color visual thresholds for perceptibily ($PT_{00} = 0.81 \Delta E_{00}$ units) and acceptability (AT₀₀=1.77 ΔE_{00} units). The results of this study showed higher ΔE_{00} (ΔE_{00R} , ΔE_{00C} , ΔE_{002P}) and lower CAP₀₀ (CAP_{00C} and CAP_{00P}) for cavities with 1 mm wall thickness (p < 0.05), especially for VU (p < 0.05). Restorations matched the colour of the control composite better in the peripheral area than in the central area (CAP_{00C} > CAP_{00P} ; p < 0.001). Overall, ΔE_{00} means were above the colour perceptibility and acceptability thresholds. Positive CAP₀₀ values were found for VU and OM. In conclusion, wall thickness negatively influenced the CAP₀₀ of single shade composites, as 1-mm-thick cavity walls resulted in lower CAP₀₀ values for both composites, especially for VU. Colour matching ability was structure and material dependent, and clinicians should be aware of this fact when using single shade composites.

Keywords: CIEDE2000; color adjustment potential; color perception; composite resins; dental cavity preparation.